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Semi-Quantitative Assessment on Winyah Bay's Microplastic Contamination Levels

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Master's Thesis

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Abstract

Although there is much concern globally regarding microplastic contamination in aquatic environments, there is little documentation on microplastic contamination concentrations within South Carolina inland waters and coastal areas. Specifically, Winyah Bay, SC should be of high priority for microplastic contamination evaluations as it is the third largest drainage basin (47,060 km^2) and the second largest (157 km^2) estuarine system on the east coast of the United States. Today, the general influencing physical factors on microplastic transport are mostly known by the scientific community, however, transport and deposition patterns of microplastics heavily vary by location depending on various physical, chemical, and ecological factors. We tested several different microplastic extraction techniques for Winyah Bay's sample type and we advanced the methodology to be more efficient. Bottle and grab samples were taken from surface waters and surface sediment, respectively, within Winyah Bay and its associated rivers. Microplastics were extracted from water samples through plankton nets and sieves with hydrogen peroxide for organic matter digestion as needed. We found that filtering water samples through a sieve reduced processing contamination in comparison to filtering through a plankton net. Additionally, we found that extracting microplastics from muddy, thus strongly cohesive sediment samples, was more easily and efficiently accomplished using highly volatile kerosene instead of the commonly used canola oil of higher viscosity. The methodological development of extracting microplastics from these cohesive sediments was so time consuming, such that, no microplastic abundance data from sediments were produced from this study. The average (1.64 particles/L for 2016a, 6.24 particles/L for 2016b, and 0.31 particles/L for 2017; >63 µm, 28-50% particle recovery rate) is comparable to other

microplastic abundances across the surface waters (~0.3 m) of the coastal eastern United States (i.e., Georgia: 2 particles/L and some Florida sites: 1-5 particles/L). Oppositely, the average is not as comparable to other sites of the coastal eastern United States (i.e., other Florida sites: 6-20 particles/L and Chesapeake Bay: 1-560 g/km^2). Additionally, microplastic fibers made up 90% of the total microplastics collected from surface water samples in this study which is comparable to the domination of fibers also found in Georgia and Florida studies. This study adds to the demonstration that there is a consistent challenge in microplastic literature to compare between studies as each study uses different methods. Our data suggests that microplastic abundances in Winyah Bay, SC are influenced by precipitation ($r = .411$, $p = .030$) and tides (i.e., salinity ($r = .402$, $p = .034$), density ($r = .422$, $p = .025$), and tidal range (.400, $p = .035$)).

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Introduction

Plastic contamination. Over the past half-century, global plastic production has increased from 1.5 million tons to 280 million tons (Plastics Europe, 2016). Such an increase in production has accelerated the distribution of plastic contamination to a variety of environments throughout the world (Barnes et al., 2009; Ryan et al., 2009; Moore, 2008) and has made plastic the dominant type of anthropogenic debris found in the marine environment today (Barnes et al., 2009). Additionally, with half the world's population living within fifty miles of the coast, the potential for plastic contamination to reach the marine environment via river transport, wastewater input, and wind is high (Moore, 2008). While the high durability and high resistance to degradation of plastics are beneficial to societal usage, these characteristics are damaging when released into the environment. A majority of all plastic waste is disposed in landfills, a small portion is recycled and about 10% ultimately end up in the ocean (Barnes et al., 2009; Moore, 2008). Unfortunately, plastic degradation rates are extremely slow (Müller et al., 2001), especially under aquatic conditions (Andrady, 2011). Plastic persistence causes environmental hazards for all organisms so much that entanglements, injuries, and deaths to marine birds, mammals, fish and reptiles are frequently reported (Derraik et al., 2002; Gregory, 2009; Lozano and Mouat, 2009). As a result, many state and federal agencies and non-governmental organizations have established procedures for the management and study of plastic contamination issues (Derraik et al., 2002).

Microplastic contamination. As plastics fragment in the environment over time, there is a decrease in the average size of plastic litter (Barnes et al., 2009). Plastic debris particles (fragments, pellets, fibers, etc.) that are less than 5 mm in size (Arthur et al., 2009)

are referred to as “microplastics”. Microplastics are either derived from the physical degradation of macroplastic debris (e.g., plastic bottles, fishing line, plastic bags, etc.) or are manufactured for abrasive-type products like some personal care products (Derraik et al., 2002; Browne, 2015; Thompson, 2015). In recent years, microplastic-related environmental concerns have increased as they have been shown to adversely affect marine organisms, including seabirds (Tanaka et al., 2013), blue mussels (Moos et al., 2012), fish and crustaceans (Laist, 1987). Due to their small size and low density, it is believed that microplastics easily become bioavailable to organisms within the food web (Andrady, 2011; Cole et al., 2011). Plastic ingestion can be extremely dangerous as plastics contain additive chemicals (e.g., halogen stabilizers, antioxidants, ultraviolet absorbers, and biological preservatives) and tend to sorb persistent organic pollutants (POPs) (e.g., polychlorinated biphenyls (PCBs) and polycyclic aromatic hydrocarbons (PAHs)) from the environment (Rochman et al., 2013). Ingestion of such chemicals may lead to endocrine disruption within organisms (Teuten et al., 2009).

Sources of microplastic contamination. Microplastics have been reported in many marine environments worldwide including surface water (Lusher et al., 2015), beach sand (Cole et al., 2011, Yu et al., 2018), deep-sea sediment (Lozano and Mouat, 2009; Cauwenberghe et al., 2013), polar glaciers (Lusher et al., 2015; Barnes et al., 2010), mid-ocean islands (Sul et al., 2009), coastal pelagic ecosystems (Doyle et al., 2011), and even in remote protected areas (Turra et al., 2014; Yu et al., 2018). Type and abundance of microplastics vary across areas (Moore, 2008; Martinez et al., 2009) and depend on the contributing plastic sources. It is assumed that 80% of marine debris, including microplastics, are from continental sources such as wastewater, clothes driers, and runoff

from solid waste disposal, landfills, roads, and agricultural land (Andrady, 2011; Allsopp et al., 2006). Moore et al. (2008) showed that two Los Angeles (California, USA) rivers alone release over 2 billion plastic particles into the marine environment over a 3-day period (Moore, 2008). The remaining 20% of marine debris, including microplastics, are assumed to be from ocean-based sources through accidental spills, indiscriminate littering or dumping from commercial fishing, recreational boaters, merchant, military and research vessels, or offshore oil and gas platforms and exploration (Allsopp et al., 2006). However, studies vary in findings on the relationship between spatial variation in microplastic concentration with the population density or proximity to industrial areas or sewage treatment plants (Ryan et al., 2009; Doyle et al., 2011; Yu et al., 2018; Mani et al., 2015). Variation such as these could also be due to the variety of methods used to characterize such a widely under-researched topic.

Continental microplastic transportation dynamics. Dris et al. (2015) provides a systematic approach to understanding continental microplastic dynamics including land-based sources and aquatic pathways. Continental microplastic transport can occur through many ways that can be summed up into three categories of transport: wind, runoff, and river. The transport of microplastics by wind can begin at sites containing clothes dryer machines, plastic incinerators, landfills, etc. Transport of microplastics by wind covers the largest amount of geographic area between all three categories of transport and even has potential to exchange microplastic between watersheds as the particles drift over the land. Microplastic particles on the land that is not lifted by the wind will be transported to rivers or the ocean through runoff from the land. Microplastics transported by runoff from the land begin at sites like agricultural ecosystems where plastic-containing soils were added,

domestic and industrial dumping areas, and even more broadly, areas that experience atmospheric fallout of plastics (Dris et al., 2015). Like wind, runoff from the land also covers a wide geographic area but, unlike wind, it is limited to the area of its own watershed. Lastly, plastics can be transported by rivers and are input through wastewater treatment plants, combined sewer overflows, direct dumping, river traffic, and atmospheric fallout. Microplastics transported by rivers are limited to a focused geographic area (e.g., only the area of the river course) in comparison to the area-wide transport by wind and runoff over/across the land. During riverine transport, microplastics may fall out of suspension depending on their densities and deposit into riverine sediments or may even be transported over longer distances in suspension and settle later into estuarine, coastal, or oceanic sediment. Besides microplastic density, vertical fluxes of microplastics also depend on the surrounding hydrodynamics (Law et al., 2010), flocculation of material (Nor et al., 2014), or due to an increase in the density of the microplastic from biological growth on the particle (Barnes and Milner, 2004).

Density of microplastics. Microplastics have been found worldwide at the surface, middle, and bottom of the water column, and in sediments (Cole et al., 2011; Hanvey et al., 2017; Claessens et al., 2011; Lima et al., 2014; Browne et al., 2011). Once microplastics are released into aquatic environments, their transportation and dispersion are controlled by the density of the plastic, the density of the water mass they are in, the presence and intensity of river flow and ocean currents, winds, tides, and the interaction of these factors together (Barnes et al., 2009; Allsopp et al., 2006; Ng et al., 2006). The density values of polymers range from 0.9 to 1.6 g/cm^3. Typically, plastics that are more dense than freshwater (1.000 g/cm^3) and seawater (1.020-1.070 g/cm^3) will sink to the sediments (e.g.,

polyvinyl chloride (1.14-1.56 g/cm^3) and polyethylene terephthalate (1.32-1.41 g/cm^3)) while plastics less dense will float in the water column (e.g., high-density polyethylene (0.95-0.97 g/cm^3) and polypropylene (0.90-0.91 g/cm^3)). However, there are several reasons that cause the above statement to be not as straight forward. For example, under tidal or turbulent influence, more dense plastics may remain in suspension (Browne et al., 2010). Additionally, the larger the surface area of the plastic piece, the higher the chance the plastic will be brought into suspension by the river or tidal flow (Browne et al., 2010). Also, as biomass accumulates on plastic particles, the density of the particle increases (Lima et al., 2014). Lastly, if flocculation of particles occurs, the plastic will sink into the bottom sediment (Cauwenberghe et al., 2015).

Methodologies for microplastic extraction. Methodology for microplastic extraction from samples varies across and within different sampling matrices: sediment, water, and soil. Sample collection methods vary between sieves, plankton nets, and grab samples. Sample processing methods vary between separation through density differences, oil by taking advantage of oleophilic properties, or through density/flow differences using elutriation, digestion of organic matter by acids, bases, or enzymes, and lastly vacuum filtration. Each method reports different microplastic extraction efficiencies leaving comparisons between studies challenging or even impracticable. Even more so, there are a wide variety of methods for extracting microplastics from sediment samples, which only started to appear in the scientific literature 15 years ago (Miller et al., 2017). Additionally, there are a variety of sediment types (e.g. beach and riverine sands, mangrove and estuarine muds, municipal soils, and deep-sea sediments) investigated as well as a variety of locations and depths sampled (Miller et al., 2017). While the density separation method

using sodium chloride (NaCl) is one of the most common to extract microplastics from sediment samples (Miller et al., 2017), recovery rates can be as low as 52% depending on the type of plastic desired for extraction (Quinn et al., 2017). The density of plastic ranges between 0.9 to 1.6 g/cm^3 depending on the type of plastic, which can make microplastic extraction through density separation with NaCl inefficient (~1.2 g/cm^3) (~50% of studies use this method) (Hanvey et al., 2017). Other solutions used for density separation include sodium iodide (NaI) (Claessens et al., 2013), zinc chloride ($ZnCl_2$) (Horton et al., 2017), zinc bromide ($ZnBr_2$) (Quinn et al., 2017), and lithium metatungstate (Masura et al., 2015) which are all more dense than NaCl, however, they are also more expensive than NaCl. Other separation methods that do not involve density differences are staining microplastics with Nile Red (NR) acetone solution (Maes et al., 2017), lifting microplastic particles with elutriation (Claessens et al., 2013), exploiting the oleophilic properties of microplastics by using canola oil (Crichton et al., 2017), and a pressurized fluid extraction using methanol and dichloromethane (Fuller et al., 2016). All methods were investigated by Miller et al. (2017) and they listed the advantages and disadvantages of each. Crichton et al. (2017) was listed to have one of the fewest disadvantages to its method of exploiting the oleophilic properties of microplastics by using canola oil. Two disadvantages were identified which were that (1) this method involves an additional clean up step for allowing plastic composition identification through FTIR/Raman and (2) this method was not confirmed for applications on water samples. The advantages of Crichton et al. (2017) method far outweigh the disadvantages as the method is fast, inexpensive ($4.50/L), has high recovery rates (96.1%) even with high-density plastics, and it's applicable to fine sand and silt. This method is specifically useful on the black, jelly-like muds in this project because the oil

extracts microplastics from a sample and leaves the fine sand and silt sediment and organic particles behind.

Current study's motivation. The motivation of this study is to shed light on the microplastic contamination levels within Winyah Bay, SC. Winyah Bay is surrounded by a flat southeastern US coastal plain with low human population density that provides a variety of coastal habitats for wildlife, contains important resources for fishery across the region, and contributes largely to the region's tourist industry (Georgetown County, 2018). The focus of this study is to determine the levels of microplastic contamination in Winyah Bay and to assess and improve the laborious methodology of microplastic extraction from Winyah Bay's distinct sample matrices.

Regional Setting and Approach

Study area. Few studies have been conducted that document microplastic contamination levels within South Carolina inland waters and coastal areas. The study area of this project is Winyah Bay, a coastal estuary into which the Waccamaw River, Atlantic Intracoastal Waterway, Pee Dee River, Black River, and Sampit River discharge. According to U.S. Census Bureau 2017 data, Winyah Bay's total watershed (47,060 km^2) (Kim et al., 2008; North Inlet-Winyah Bay National Estuarine Reserve, 2009), including up to the start of the Yadkin's River in North Carolina, is home to 3 million residents. Forested land covers half the basin (North Carolina Environmental Education, 2018). Originating from the Blue Ridge and spanning over the Piedmont, Sandhills, and Coastal Plain, the basin contains a wide variety of habitats home to many rare species including 38 aquatic animals (North Carolina Environmental Education, 2018). Even though these rivers

and Winyah Bay are surrounded by little development (13% of the watershed) (Figure 1) (North Carolina Environmental Education, 2018; Allen et al., 2014), they are still prone to microplastic invasion as the bay receives agricultural (one-third of the watershed) (North Carolina Environmental Education, 2018), industrial, and domestic waste runoff from its watershed.

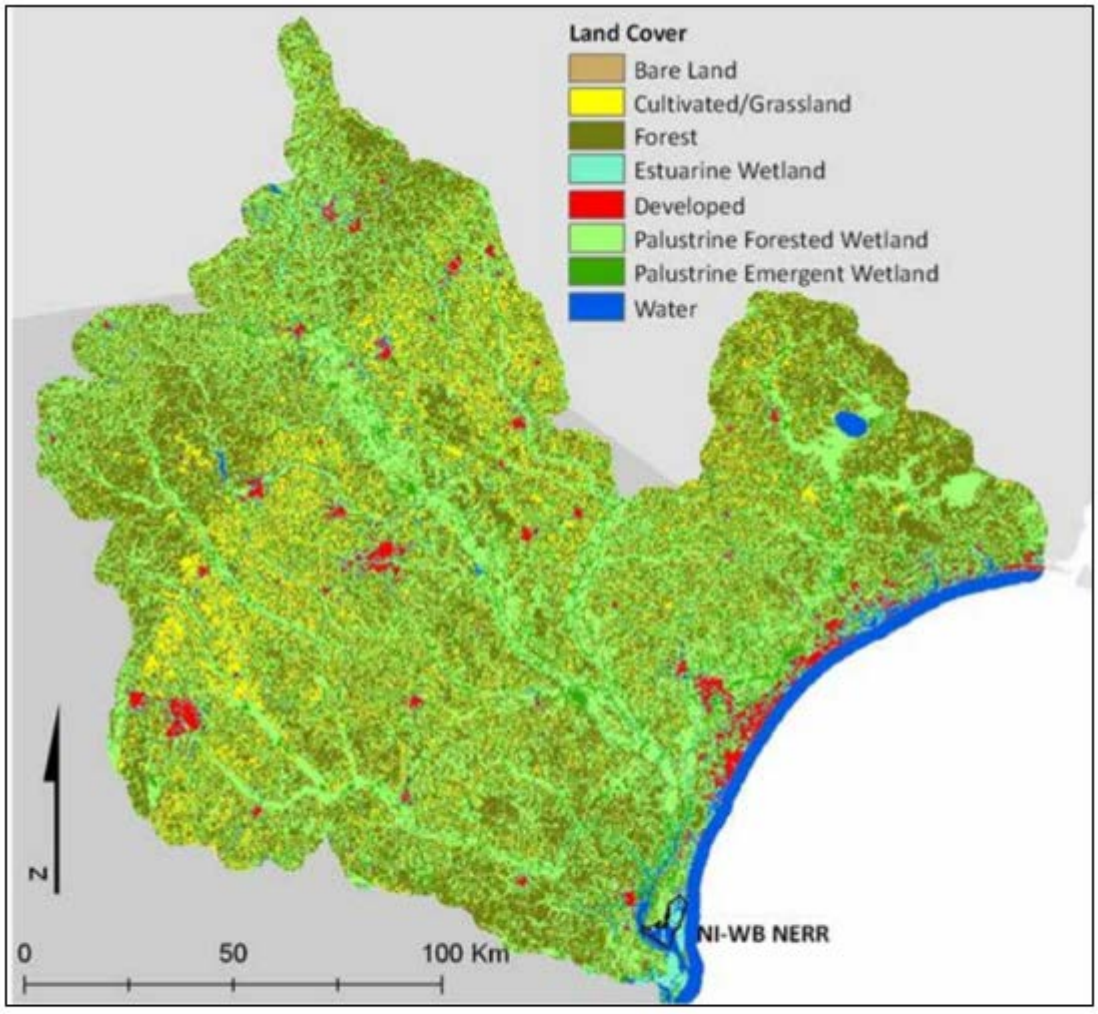

Figure 1. Map of the Winyah Bay watershed land use and land cover of 2014. Colors represent different land use and coverage indicated in key. Taken from Allen et al. (2014).

Characteristics of Winyah Bay. Winyah Bay on average is only 4.2 m deep, with max water depth of 6.5 m in the shipping channel originally dug to 8.2 m depth (Kim et

al., 2008). Winyah Bay experiences semidiurnal tides and is a partially mixed estuary with a salt front (i.e., a fresh and saltwater boundary causing a steep salinity gradient) whose geographic location is very dependent on the freshwater discharge rates (Kim et al., 2008). Winyah Bay has an annual mean freshwater discharge of approximately 557 m^3/s (Patchineelam et al., 1999). Under high river discharge the salt front moves down to the estuary mouth, but under low river discharge the salt front moves upstream as far as 55 km (Blood and Vernberg, 1992; Patchineelam and Kjerfve, 2004). Tides have been recorded to penetrate about 100 km into the Waccamaw River at amplitudes of 0.1 to 0.3 m (Army Corps of Engineers, 1977).

Winyah Bay's tributary rivers. Of Winyah Bay's tributary rivers, the Pee Dee is the main influence on the Winyah Bay system as it delivers 52% of the total discharge that Winyah Bay receives (Kim et al., 2008). The Pee Dee River reaches about 705 km from Winyah Bay into North Carolina and is home to many migratory fish, shellfish, black bears, bald eagles, swallowtail kites, and some rare and endangered species (Kim et al., 2008; American Rivers, 2016). Duke Energy's Tillery Dam has impacted the flow of the Pee Dee River since 1928 and currently releases 9.3 m^3/sec except for 10 weeks in the spring when it releases 20.5 m^3/sec for spawning season (American Rivers, 2016). This discharge fluctuation caused by the Dam may have an impact on the total river discharge that Winyah Bay receives. The Black River joins the Pee Dee before the Pee Dee connects with Winyah Bay. The Waccamaw River and the Atlantic Intracoastal Waterway also join before connecting with Winyah Bay. Lastly, the Sampit River joins Winyah Bay south of the Pee Dee and Waccamaw mouths just after it flows by Georgetown Harbor and connects to the Bay (Figure 2).

Figure 2. Map of USGS hydrologic unit catchment area of the major rivers (dark blue lines) and the watershed (blue-green shading) of Winyah Bay (dark blue shading). Gray shading is developed areas, greens are state lines and light blue is the ocean. Duke Energy's Tillery Dam location is indicated by the red line on the Yadkin-Pee Dee River. Modified from (North Inlet-Winyah Bay National Estuarine Research Reserve, 2009).

Focus and objective. This project provides a semi-quantitative assessment on microplastic concentrations in Winyah Bay. Additionally, the collected samples underwent numerous scientific literature-searched preparation methods to reveal the most efficient, clean and comprehensive way to extract microplastic particles from these site-specific samples. This study focuses on microplastic concentrations within sub-surface water samples, presumed to represent a snapshot of microplastic contamination in the area. The outcome of the project provides a new data set to microplastic contamination levels in and around Winyah Bay, SC, and some of the potential natural mechanisms that might control microplastic abundance.

Materials and Methods

a. Sample collection

Sampling sites. The sampling sites of this project were located at river mouths (Waccamaw, Pee Dee, and Sampit rivers), around Goat Island, i.e., Georgetown harbor, within the lower Sampit river, and south of Rabbit Island in the neck of Winyah Bay (Figure 3). From July to October 2016, water surface samples (~5-15 cm water depth) were taken from Winyah Bay to look for changes in microplastic abundance, type, and distribution over seasons. From September to November 2017, samples were taken again along with sediment surface samples (~20 cm thick) to look for spatial changes in microplastic abundance and type. Temperature and salinity were measured by an YSI 6600 V2-4 data sonde at the water surface (~5-15 cm water depth) and bottom directly after taking each sample. Density values were calculated from temperature and salinity using a density calculator at csgnetwork.com and 10.1325 decibars as pressure (CSG, 2018). River

discharge and tidal range (calculated from gauge maximum and minimum) values were taken from the United States Geological Survey from site 2135200 near Bucksport on the Pee Dee River (USGS, 2018). Wind origin, wind speed, and precipitation were taken from Weather Underground at the Friendfield weather station (#KSCGEORG22) (Weather Underground, 2018).

Figure 3. Map of Winyah Bay, tributary river mouths and project field sites during 2016 and 2017.

Water sample collection in 2016. Ebb tidal sampling was conducted to see how microplastic contamination distribution changed as brackish water followed by fresh water left the river mouth. Water samples for microplastic analysis were collected from the beginning to the end of the ebb tide every 30-45 minutes (~10 samples). Max ebb current velocities and tidal heights between the summer and fall season within both the Waccamaw and Pee Dee river range from 0.6-0.8 m/s and 1.1-1.3 m, respectively (USGS, 2018). River surface water sampling included obtaining grab samples of 6.5 L of surface water about 15 cm below the water surface for microplastic analysis and simultaneous water parameter measurements at the same location. The water collection jar held 0.94 L, therefore, the collection jar was filled 7 times with surface water to obtain 6.5 L of water. To do this, the cap was left on the bottle until under the water surface and then opened, filled up completely, closed underwater, lifted above water, opened and poured over the nets 7 times. The sample was first poured through the 330 μm net which drained into the 10 μm net. After a sample finished filtering through the 330 μm net, the net was rinsed with a full water bottle dispenser of Milli-Q water by pouring the water down the inside of the 330 μm net. After the rinse, the 330 μm net assembly was carefully removed from being stacked inside the 10 μm net and the 330 μm cod end was removed and the contents transferred to a glass collection jar. The 330 μm cod end was rinsed 10 times with Milli-Q water into the collection jar and then the jar was closed. After the sample finished filtering through the 10 μm net, the net was rinsed with a full water bottle dispenser of Milli-Q water by pouring the water down the inside of the 10 μm net. After the rinse, the 10 μm cod end was carefully removed from the 10 μm plankton net and the contents transferred to the same glass collection jar used previously for the 330 μm cod end collection. The 10 μm cod end was

rinsed 10 times with Milli-Q water into the collection jar and then the jar was closed and stored for lab processing.

Sterile techniques for 2016 water samples. Before taking collection jars into the field to collect water samples, the jars were washed with tap water and soap to remove microorganisms and then rinsed with Milli-Q water three times to remove any remaining microplastics from the tap water and soap. Before each sample collection, plankton nets and cod ends (10 and 330 μm) were rinsed with Milli-Q water. For rinsing, plankton nets were flipped inside out and rinsed from top to bottom and then was scrubbed against itself to remove any materials stuck in between the net pores. This was performed 3 times and then the nets were pulled back inside and rinsed again by pouring 3 water bottle dispensers full of Milli-Q water. The cod ends were wiped out with a cotton towel or paper towel and then rinsed 3 times by filling it with Milli-Q water halfway and swirling it around then dumping the rinse out into a bucket or the river. The reason the cap was left on the bottle until under the water surface and then opened, filled up completely, closed underwater, lifted above water, opened and poured over the nets was to minimize microplastic contamination from the water surface microlayer (~1 mm). In using this methodology, however, there is no way to solely sample the surface waters since the sampling jar was dipped into the water through the surface microlayer causing mixing. Additionally, while processing through the plankton nets, samples were protected from atmospheric contamination (Dris et al., 2015) by covering the 330 μm net opening with aluminum foil or printer paper.

Inconsistencies in 2016 water sample collection. Some samples were processed through the net directly in the field, while others were processed through the net after

sampling under more controlled conditions in the CMWS warehouse. Change in preparation procedure was necessary when samples collected in the field took hours to drain through the net, therefore, other samples needed to be stored and filtered through the net another time. When those samples were filtered through the net back at the CMWS warehouse, they were only filtered through the 10 μm net rather than both 330 μm and 10 μm like in the field.

Water sample collection in 2017. Water samples (~5-15 cm water depth) and sediment surface samples (~20 cm thick slices accounting for 2-3 kg of material) were collected from September to November 2017 to look for spatial changes in microplastic abundance and type within Winyah Bay and its tributary rivers (Figure 3). In the samples from 2016, the 10 μm net clogged rapidly from fine-grained sediment and organic particles resulting in hours of draining of 6.5 L sized water samples. To resolve this problem and collect more water per sample, a sieve with a larger mesh size was used (63 μm). With a clean glass jar, about 20 L (with a 0.94 L collection jar filled 22 times) of river surface water was grabbed the same way the 2016 samples were grabbed and poured through a 63 μm sieve. Before each sample collection, the sieve was rinsed carefully several times by running Milli-Q water through the sieve and using a 500 mL squirt bottle for pressure to push out any stuck particles. After collection in the sieve, the sample was rinsed with Milli-Q water and pushed to one spot on the sieve for easy transfer back into the collection jar. A series of rinses were performed consistently between samples on the sieve to maximize sample collection and then rinsed into the same sample jar. The jar was then shut, labeled and stored for later processing.

Sediment sample collection in 2017. Surface sediment samples were collected with

a stainless steel ponar grab sampler at the same locations as the 2017 surface water samples (Figure 3). Surface sediment samples were stored in tinfoil pans and topped with aluminum foil. About 2-3 kg were collected per sample. The pans were rinsed with Milli-Q water before using.

b. Sample processing

Processing 2016 water samples. Surface water samples taken in 2016 were vacuum filtered. Samples required between 3-12 filters depending on the amount of suspended material within the sample. The filters were placed in Petri dishes in a desiccator for 24 hours with the Petri dish lid slightly skewed, yet still protecting the sample from air contamination within the desiccator. After drying, the Petri dishes were removed from the desiccator, closed, and stored at room temperature for further quantitative and qualitative analyses. Due to excessive sediment on filters, counting samples was difficult without further reduction of sample material. Therefore, samples were rehydrated with Milli-Q water and put in an ultrasonic bath for 10 seconds to break cohesion between particles. Afterward, the samples were run through a 63 μm sieve, and microplastic particles were collected from the sieve as described above. The samples were then vacuum filtered using only one filter per sample. Procedural blanks using Milli-Q water were regularly (every 3 days) processed to account for any possible contamination that may occur during extraction of microplastics from the water samples.

Processing water samples of 2017. Surface water samples taken in 2017 with a 63 μm mesh size sieve were vacuumed filtered. Samples required between 1-5 filters to process depending on the amount of organic material within the sample. Visual

identification and quantification of microplastics was difficult due to large amounts of organic matter and an additional separation step was needed to fully extract microplastics from the matrix. Organic matter digestion was required only on 2017 samples because of the larger sample volume in comparison to 2016 samples. Due to safety issues related to handling some of the chemicals involved in acid, alkaline, and enzyme digestion procedures and as an affordable approach, the project used a 10% hydrogen peroxide (H_2O_2) solution, a commonly used solution to digest organic matter (Lusher et al., 2017). Hydrogen peroxide also digests nylon fibers (Claessens et al., 2013), therefore, it is expected that the outcome of the data will underestimate nylon fibers, and thus total microplastic counts. Samples were soaked in 20 mL of 10% hydrogen peroxide solution in a 400 mL beaker covered with aluminum foil for 5-6 days under a hood. Most studies involving organic matter digestion use 30% hydrogen peroxide solution (Courtene-Jones et al., 2017) to digest animal tissue. Since the study does not involve animal tissue in the samples, 30% is an unnecessary solution strength and instead only 10% hydrogen peroxide was used. After digestion of the organic matter, samples were diluted with Milli-Q water up to the 300 mL mark on the beaker and then vacuum filtered onto a new filter paper. Old filter papers were picked out of the beaker with forceps and rinsed with Milli-Q water allowing the rinses including potential microplastics to fall into the sample beaker. Samples were then vacuum filtered on to one filter per sample. Procedural blanks using Milli-Q water were regularly (every 3 days) processed to account for any possible contamination that may occur during extraction of microplastics from the water samples.

Processing sediment samples of 2017. A kilogram of the wet sediment sample plus 50 mL of Milli-Q water was put in an ultrasonic bath for 20 seconds to release the sticking

particles from cohesion and into suspension. A gram of Calgon was added to the sample to help break it up even further. The Calgon was inspected under a microscope before added to the sample for microplastic contamination. The sample was then left on a shaker table overnight and diluted the next day with 5 gallons of Milli-Q water. The diluted sample flowed easily through a 63 μm sieve and about 50-200 grams of the sample was left over after sieving. Next, to remove the organic matter within the sample that might be attached to microplastic particles, 7 mL of 30% hydrogen peroxide was added and the sample was brought up to about 5 cm from the top of the container with Milli-Q water creating an even more diluted solution. The sample was left on the shaker table overnight and the next day was diluted with 3 gallons of Milli-Q water and sieved through a 63 μm sieve. With most of the clay extracted and some of the organic material digested, the sample was ready for oil extraction. During my trials of Crichton et al. (2017) method, I found the canola oil was too viscose to vacuum filter completely through a 1 μm pore-sized filter paper. Even different filter types (e.g. cellulose-nitrate, glass fiber, and polycarbonate) did not help vacuum filter the thick canola oil. In the next trial, Crichton et al. (2017) methods were used except with highly volatile kerosene (i.e., lamp oil) instead of canola oil since kerosene is less viscose than canola oil and it can be evaporated quickly. Samples were then vacuum filtered on to three filters per sample.

Final processing steps for all samples. All samples were vacuum filtered through a 47 mm, gridded, cellulose-nitrate filter with a 1.0 μm pore size (GF/B, Whatman, USA). Aluminum foil was used to cover the filter cup to protect the sample from air contamination in the lab. Once filtered, the filter cup was rinsed three times. Filters were carefully transferred from the filter apparatus to a Petri dish with forceps and then covered with a

lid. The filters were then dried in a desiccator for 24 hours with the petri dish lid slightly askew, yet still protecting the sample from air contamination within the desiccator. The filters were considered dry when the filter paper was no longer soft, which was seen very well underneath a dissecting microscope. If the paper was still wet then most likely the plastics were still wet and the hot needle test (see the below section) would not work on the plastics during counting. After drying, the petri dishes were closed, taken out of the desiccator and stored at room temperature for further quantitative and qualitative analyses. A brief review of all the steps for sample collection and processing can be viewed in Figure 4.

c. Microplastic identification

Equipment. Two different microscopes were used in this study to quantify and identify microplastics on samples: a Dino-Lite Microscope (AD4113ZT Dino-Lite Premier, 20x~50x, 200x magnification) and a Carl Zeiss Stereo Microscope (Stemi 2000/KL 200 LED). The dissecting microscope was primarily used for identifying microplastics because it gives a very clear view of the particles on the filter paper. The Dino-Lite microscope was used to measure the size of microplastic particles and take photos. The size of each particle type was measured differently where the length is measured for fibers, but the longest width is measured for fragments, film and foam. Depending on the size of the plastic particle, magnifications varied from 50x-220x, but most microplastics were viewed at 206x magnification. Microplastic particles under 0.063 mm were too small to perform identification methods, therefore, they were not included in this study.

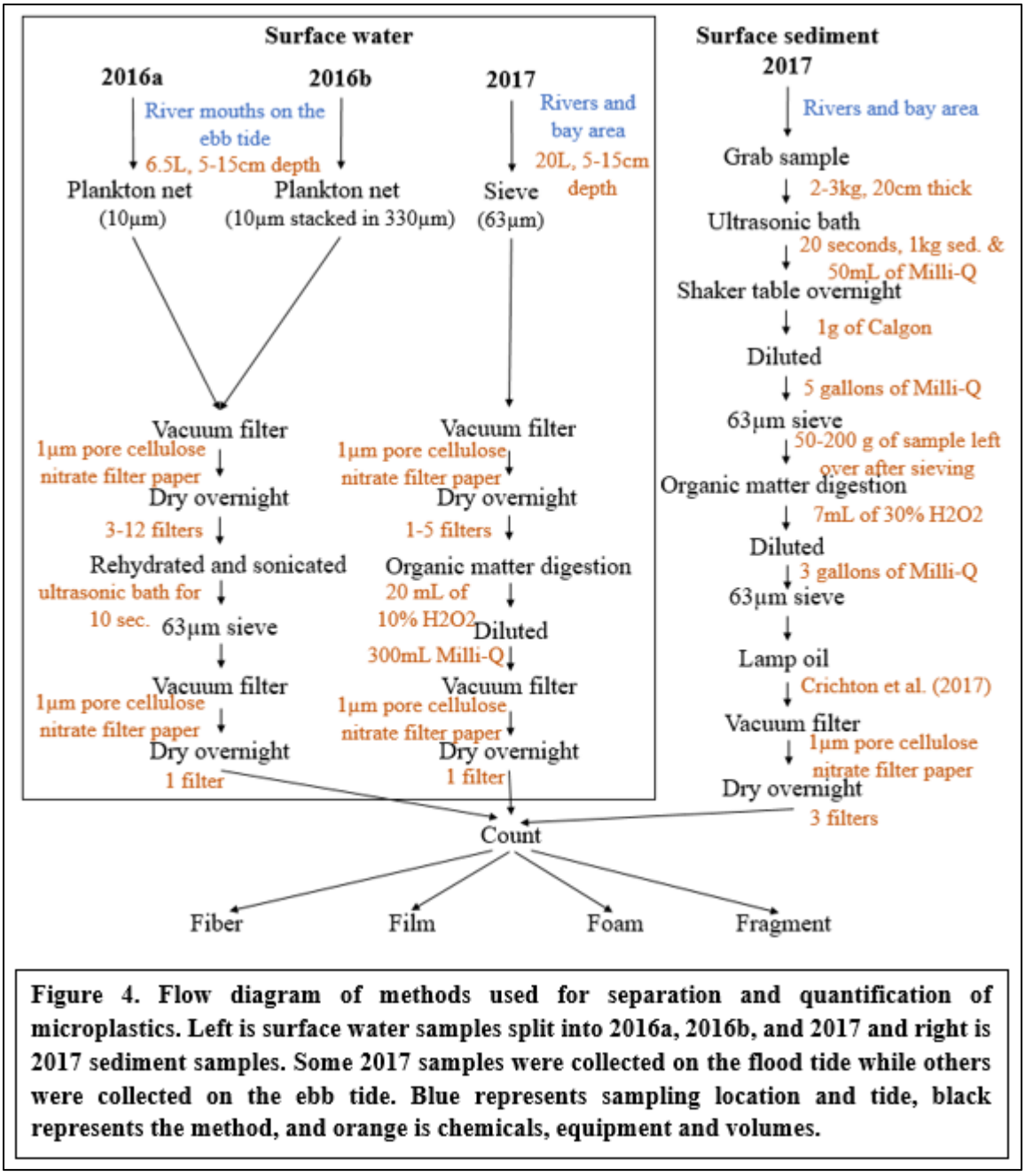

Figure 4. Flow diagram of methods used for separation and quantification of microplastics. Left is surface water samples split into 2016a, 2016b, and 2017 and right is 2017 sediment samples. Some 2017 samples were collected on the flood tide while others were collected on the ebb tide. Blue represents sampling location and tide, black represents the method, and orange is chemicals, equipment and volumes.

Guidelines to microplastic identification. Identification of microplastics was performed using Hidalgo-Ruz et al. (2012) and Barrows et al. (2017) Marine and Environmental Research Institute (MERI) Guide to Microplastic Identification (Barrows et al., 2017) (Table 1). First, filters were examined in a zig-zag manner following the grid lines starting from left to right then back right to left from the top of the filter to the bottom.

When a particle appeared in the view of the microscope, the visual variations between plastics and non-plastic materials (i.e., algae, salt, sand, shells, microorganisms, paint, and aluminum foil) were used to reduce error (Table 1). Beyond visual variation, microplastic particles were identified by prodding pieces, examining textures, picking through high debris loads, and using the hot needle test (Table 1) (Barrows et al 2017). Due to an unexpected incident at the side of the collaborator we were unable to complete the intended Fourier Transform Infrared (FTIR) analysis on probable microplastic pieces. The hot needle test was used because is the next best approach before FTIR as it can help distinguish between a plastic particle and organic matter (Witte et al., 2014).

d. Method precision and accuracy

Duplicates, background contamination, and blanks. To estimate the precision of the 2017 method, seven duplicate samples were collected. The duplicates were taken right after the sample to eliminate possible impacts due to environmental condition variability between sampling times (i.e., weather, tidal cycle, winds, tidal range, river discharge, and river current speed). Also, one blank sample was taken during each sampling day and went through the same processing that regular samples went through to account for any possible contamination that may occur. Unwanted secondary contamination may come from the air in the field, clothing from participants, the Milli-Q water used in rinsing, the jugs and squirt bottles used for dispensing the Milli-Q water, the hydrogen peroxide solution, cleaning procedures in between samples, and poor protection of samples from handling/air contamination while in the field, lab or under the microscope.

Table 1. Modified microplastic identification protocol from a combination of Barrows et al. (2017) and Hidalgo-Ruz et al. (2012).

Types of Microplastic	Description
Fiber	• Elongated and mostly uniform width throughout. Some may be frayed or split. If there's a fork, it may be algae. • Will curl and may melt with a hot needle, algae won't. • No cellular structures (e.g. no segments or lines along the fiber)
Fragments	• Hard and does not break when probed. Salt crystals will break apart and sand will sound like breaking glass. Use the hot needle test (needs very hot needle to melt the hard plastic) for confirmation, as salt and sand will not melt. • Rounded, sub-rounded, sub-angular, or angular • Usually have a haze and are not completely transparent • No cellular structures, organized lines or geometrical structures
Pellets (***in this study, pellets are fragments since not all pellets are perfectly cylindrical***)	• Hard, smooth surface and shaped as cylindrical, disks, flat, ovoid, or spheroid • Won't shatter when probed and need a very hot needle to melt. • Usually have a haze and are not completely transparent • No cellular structures (e.g. a central circle)
Film	• Flexible, may tear when probed, and will curl and may melt with a hot needle • These have a haze, color or can be transparent • No cellular structures (e.g. no organized segments, circles or dots)
Foam	• Looks like a fragment but is squishy, flexible instead of hard • Will curl and may melt with a hot needle • May tear or squish/flatten when probed • No cellular structures or animal parts

Efficiency of methods used. Method efficiency examination was performed by spiking clean Milli-Q water with known concentrations of microplastics and subjecting the water to the methods described above. Microplastics of different sizes and materials originating from previously extracted water samples during preliminary testing were used for spiking the efficiency samples. Milli-Q water was spiked with 12 microplastics per efficiency test. There was a total of two efficiency tests (i.e., 2016 using one net & 2017

using one sieve) each with 3 samples, therefore, a total of 6 efficiency samples.

e. Data analysis

Samples collected. A total of 100 samples were taken from Winyah Bay from July 2016 to November 2017 during summer and fall seasons. Of these 100 samples, 84 are water samples and 16 are sediment samples. For the initial trials, seven of the water samples and 1 kg of the GS 6 (Georgetown Silting) sediment sample was used for testing methods. Of the remaining 93 samples, 49 are 2016 surface water samples, 28 (including duplicates) are 2017 surface water samples, and 16 are sediment samples. Of the total 49 surface water samples from 2016, 27 were filtered through a 10 µm net in the CMWS warehouse and are labeled as 2016a and the other 22 were filtered through a 10 µm net stacked inside a 330 µm net while in the field and are labeled as 2016b (Table 2).

Table 2. All samples collected in the current study including the year, method and sample size.

	2016, n = 55	**2017, n = 44**
Preliminary water samples	330 µm net, n = 7	None
Water samples	2016a: 10 µm net, n = 26 2016b: 10 µm net stacked in 330 µm net, n = 22	63 µm sieve, n = 28
Sediment samples	None	Grab sampler (ponar), n =16
	Total samples, n = 99	

Statistical tests. Since the surface water samples of this study were not processed the same way, statistical analyses for each method were run separately. Water samples were grouped into 2016a for those taken with 1 net, 2016b for those taken with 2 nets, and 2017 for those taken with a sieve. A two-factor analysis of variance (ANOVA) was run to

determine if there were differences in microplastic abundance between sites and on different sampling dates within different sampling methods. Correlations were run to measure the extent of interdependence between abundance in a given sampling method with salinity, temperature, water density, wind origin, precipitation, river discharge and tidal range. Correlations were also run to measure the extent of interdependence between microplastic type and length with water salinity, temperature and density. All analyses were done using IBM SPSS Statistics 24.0 (2016).

Results

Efficiency of methods used. The results of the method efficiency examinations are shown in Table 3. High processing contamination occurred when using a plankton net and little contamination occurred when using a sieve to filter surface water samples. The large surface area of the net requires a lot of rinsing to clean the net completely in comparison to the small surface area of the sieve requiring fewer rinses to rid it of particles. A lower retention rate was obtained when using the sieve (28%) in comparison to the net (50%). The small mesh size of the net (10 µm) keeps elongated particles (i.e., microplastic fibers) from slipping through in comparison to the larger mesh size of the sieve (63 µm). In conclusion, samples that were processed with the net seem to be an overrepresentation of the true microplastic particle levels due to processing contamination by the net, while at the same time they are not completely an overrepresentation due to the 50% particle retention rate of the method. In contrast, samples that were processed with the sieve seem to be an underrepresentation of the true microplastic contamination levels due to the 28% particle retention rate. This method comparison study revealed that we will not be able to

appropriately compare the two methods in the data analysis.

Table 3. Different methods tested on water and sediment samples and the results.

Sample Type	Method	Result
Surface water	2016 method (10 μm net), n = 3	50% retention rate, st. dev.: 0 High contamination
	2017 method (63 μm sieve), n = 3	28% retention rate, st. dev: 0.09 Low contamination
	20 mL of 15% Hydrogen peroxide digestion for 3 days n = 3	Destroyed filter paper which added material to sample solution
	20 mL of 10% Hydrogen peroxide digestion for 5 days n = 3	Dissolved half of the total sample material and filter paper was not destroyed
Surface sediment	Canola oil separation on 50 g of sample, n = 1	Filters did not completely dry after 3 days in desiccator; plastic particles were wet. 36 plastic particles in water layer 25 plastic particles in oil layer
	Canola oil separation on 1 kg of sample, n = 2	Could not push the oil layer through the filter (tried glass fiber, polycarbonate and cellulose-nitrate filters)
	Kerosene separation, n = 3	Oil easily filtered through the cellulose-nitrate filter and filters dried completely overnight

Microplastic abundance, type, length, and color. Microplastic particles were found in all samples of this study except for 3 and total of 1536 particles were collected. The average microplastic abundance for 2016a samples was 1.64 mp/L, for 2017b samples was 6.24 mp/L, and for 2017 samples was 0.31 mp/L with retention rates of 28-50% (standard deviation: 0-0.09). Of the total microplastics collected, fibers made up 90%, fragments made up 8%, film made up 1% and foam made up 1% (Figure 5). No significant correlation was seen between microplastic type and salinity, temperature or density, however, a significant difference between means was found (Table 4). Microplastic types were found to be statistically different from each other through the salinity and temperature values from the sample water (Salinity: Fiber and Frag p = .010; Temperature: Fiber & Frag p =

.000, Fiber & Film p = .000, Film & Foam p = .019, Film & Frag p = .001). For total microplastics collected, 49% were between 0.063-1 mm, 32% were between 1-2 mm, 11% were between 2-3 mm, 4% were between 3-4 mm, and 2% were between 4-5 mm, and 2% were above 5 mm (Figure 6). There was a negative correlation between microplastic length and salinity (r = -.077, p = .005), temperature (r = -.063, p = .020) and density (r = -.056, p = .040) (Table 5). Of the total microplastics collected, 32% were blue, 20% were colorless, 19% were black, 7% were red, 5% were pink, 4% were green, 3% were yellow, 2% were gray, 2% were purple, 2% were white, 2% were multicolored, 1% were orange, 0.3% were brown, and 0.3% were melted or burned before the color was recorded.

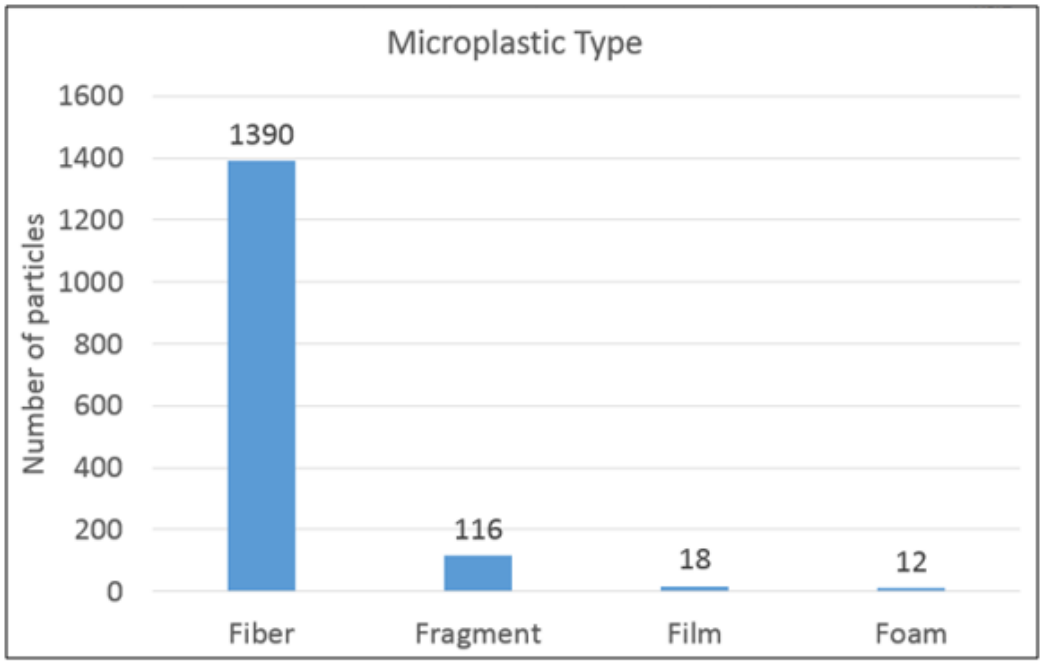

Figure 5. Microplastic types collected (i.e., fiber, fragment, film and foam). Numbers above the bars represent the total count for that microplastic type.

Table 4. ANOVA values between microplastic type and salinity, temperature, and density. ANOVA results are listed as the F-value first followed by a p-value and the degrees of freedom. Bolded values indicate means are significantly different. Post Hoc Tukey test results are listed if significance was found. Means are listed below Tukey test results.

	Salinity	Temperature	Density
ANOVA: Mp type	**F = 5.312, p = .001, df = 3**	**F = 30.137, p = .000, df = 3**	F = .701 p = .551 df = 3
	Tukey: Fiber & Frag, p = .010	**Tukey: Fiber & Frag, p = .000 Fiber & Film, p = .000 Film & Foam, p = .019 Film & Frag, p = .001**	
	Mean: Fiber = 2.704 ± .065 Film = 1.263 ± .566 Foam = 3.060 ± .693 **Frag = 1.984 ± .223**	**Mean: Fiber = 29.469 ± .059 Film = 25.906 ± .517 Foam = 28.292 ± .634 Frag = 28.064 ± .204**	

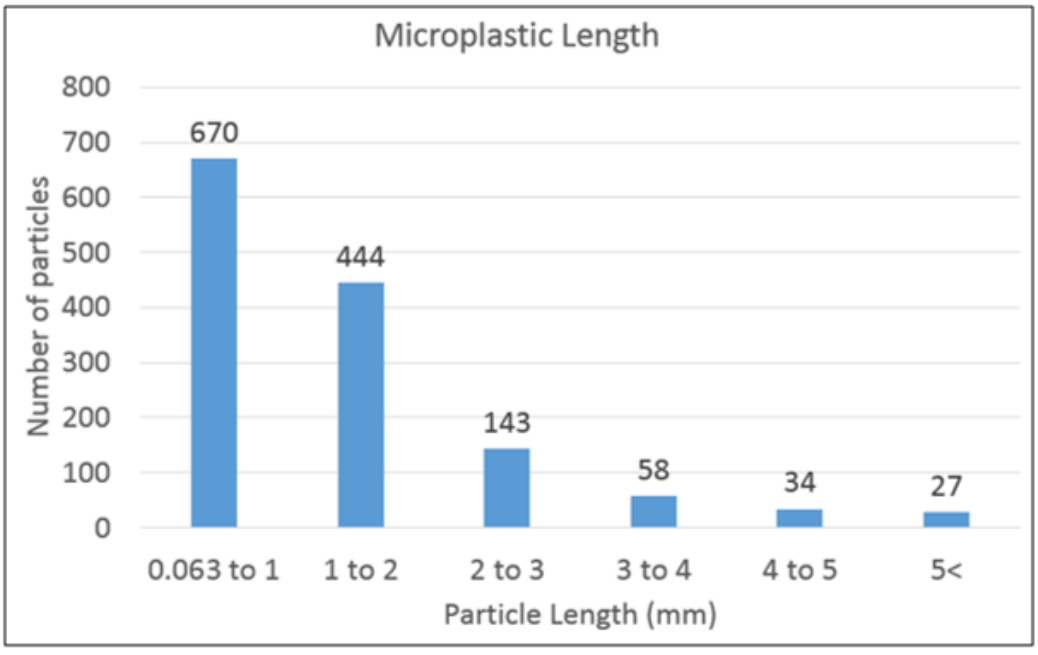

Figure 6. Microplastic sizes collected on the x-axis and number of particles counted on the y-axis. Numbers above the bars represent the total count for that microplastic type.

Table 5. Correlation values between microplastic length, salinity, temperature, and density. Bolded values indicate the correlation is significant. Correlation is significant at the 0.05 level (2-tailed) if noted with * and at the 0.01 level (2-tailed) if noted with **.

		Mp length (mm)	Salinity (ppt)	Temp °C	Density of water
Mp length (mm)	Pearson Correlation	1	**-.077****	**-.063***	**-.056***
	Sig. (2-tailed)		.005	.020	.040
	N	1357	1357	1357	1357
Salinity (ppt)	Pearson Correlation	**-.077****	1	**.230****	**.940****
	Sig. (2-tailed)	.005		.000	.000
	N	1357	1357	1357	1357
Temp °C	Pearson Correlation	**-.063***	**.230****	1	**-.115****
	Sig. (2-tailed)	.020	.000		.000
	N	1357	1357	1357	1357
Density of water	Pearson Correlation	**-.056***	**.940****	**-.115****	1
	Sig. (2-tailed)	.040	.000	.000	
	N	1357	1357	1357	1357

Microplastic abundance and environmental parameters. No significant correlations were found for either 2016a or 2016b between microplastic abundance and the variables listed in Table 6. Correlations were found between 2017 microplastic abundance and four environmental parameters: salinity ($r = .402$, $p = .034$), density ($r = .422$, $p = .025$), precipitation ($r = .411$, $p = .030$), and tidal range ($r = .400$, $p = .035$) as shown in Table 6. No significant differences were found between microplastic abundance and sample date or sample site for 2016a, 2016b, or 2017 (Table 7). An interaction was found between microplastic abundance and sampling date by sampling site for 2016a samples ($F = 5.581$, $p = .028$, $df = 1$).

Table 6. Correlation values between 2016a, 2016b, and 2017 microplastic abundance and salinity, temperature, density, wind origin, wind speed, precipitation, river discharge, and tidal range. Correlation values are listed first followed by a comma and p-value. Bolded values indicate the correlation is significant.

	Sal.	Temp.	Den.	Wind Speed	Precip.	River Dis.	Tidal Range
2016a Mp	.112, .586	.268, .186	-.010, .959	.215, .291	-.249, .219	-.296, .142	.248, .221
2016b Mp	.165, .462	.082, .717	.156, .488	.074, .744	-.019, .934	.094, .679	-.064, .779
2017 Mp	**.402, .034**	-.202, .303	**.422, .025**	.195, .320	**.411, .030**	.254, .192	**.400, .035**

Table 7. Two-way ANOVA values between 2016a, 2016b, and 2017 microplastic abundance and sample date, sample site, and sample date*site. Two-way ANOVA results are listed as the F-value first followed by a p-value and the degrees of freedom. Bolded values indicate means are significantly different. Post Hoc Tukey test results are listed if a significant difference was found unless there were fewer than three groups or if at least one group has fewer than two cases.

	Sample Date	Sample Site	Date*Site	Wind Origin
2016a Mp	F = 1.497 p = .235 df = 1	F = 1.185 p = .325 df = 2	**F = 5.581** **p = .028** **df = 1**	F = 1.128 p = .394 df = 8
2016b Mp	F = .100 p = .756 df = 1	F = 1.633 p = .225 df = 2	F = 3.094 p = .097 df = 1	F = 1.259 p = .332 df = 6
2017 Mp	F = 2.781 p = .084 df = 2	F = .437 p = .729 df = 3	F = . p = . df = 0	F = 1.096 p = .391 df = 5

Duplicates and blanks. Seven duplicates were taken and counted as separate samples to demonstrate the 2017 method reproducibility and the results were added to the data set. Duplicates varied from the original sample in the range 0.05 to 0.3 mp/L with an average of 0.2 mp/L (Table 8). Duplicates were only taken for the 2017 data set. Twelve blanks were taken to reveal the sample contamination load obtained during processing the sample. The first nine blanks were only taken in the lab during filtering the 2016a and

2016b samples, so they did not account for contamination from the net or in the field. The last three blanks were taken just like a 2017 sample taken in the field and processed in the lab. For all of 2016 samples, blanks varied from 0.3 to 0.75 mp/L with an average of 0.40 mp/L and for all 2017 samples, blanks varied from 0.05 to 0.15 mp/L with an average of 0.12 mp/L (Table 9). The average microplastic abundance for 2016a, 2016b, and 2017 samples was about 4X, 15X, and 2X their blanks, respectively. The blanks are comparable to the difference between samples and their duplicate and they may explain the variation observed in the duplicate experiments.

Table 8. Water samples and their duplicates listed by date, sample name and microplastic counts per 20 L (as collected), per liter, and per meter cubed. Difference between samples and their duplicate are listed as microplastic counts per 20 L (as collected), per liter, and per meter cubed. Averages of the differences are at the bottom of the table.

Date	Sample	Mp/ 20L	Mp/ L	Mp/ m^3	|Sample – Duplicate| Mp/20L	|Sample – Duplicate| Mp/L	|Sample – Duplicate| Mp/m^3
9.9.2017	GS8	7	0.35	350			
9.9.2017	GS8 dup.	8	0.4	400	1	0.05	50
9.16.2017	WY2	5	0.25	250			
9.16.2017	WY2 dup.	11	0.55	550	6	0.3	300
9.23.2017	WY5	3	0.15	150			
9.23.2017	WY5 dup.	6	0.3	300	3	0.15	150
9.23.2017	GS10	5	0.25	250			
9.23.2017	GS10 dup.	10	0.5	500	5	0.25	250
9.23.2017	GS11	4	0.2	200			
9.23.2017	GS11 dup.	10	0.5	500	6	0.3	300
9.23.2017	GS12	2	0.1	100			
9.23.2017	GS12 dup.	4	0.2	200	2	0.1	100
9.23.2017	GS13	12	0.6	600			
9.23.2017	GS13 dup.	10	0.5	500	2	0.1	100
				Average:	**3.57**	**0.18**	**178.57**

Table 9. Microplastic abundance found in blank samples listed by date, blank number and microplastic counts per 6.6 L or per 20 L (as collected), per liter, and per meter cubed.

Date	Blank	Mp/6.6L	Mp/L	Mp/m^3
Aug-16	1	2	0.3	303
Aug-16	2	1	0.15	152
Aug-16	3	1	0.15	152
Aug-16	4	5	0.75	758
Aug-16	5	3	0.45	455
Oct-16	6	3	0.45	455
Oct-16	7	3	0.45	455
Oct-16	8	4	0.61	606
Oct-16	9	2	0.3	303
	average:	**2.67**	**0.40**	**404.33**
9.9.2017	10	3 (per 20 L)	0.15	150
9.16.2017	11	1 (per 20 L)	0.05	50
9.23.2017	12	3 (per 20 L)	0.15	150
	average:	**2.33**	**0.12**	**116.67**

Discussion

Microplastic in Eastern United States. The average (i.e., 1.64 particles/L for 2016a, 6.24 mp/L for 2016b, and 0.31 mp/L for 2017; >63 µm, 28-50% particle recovery rate) microplastic abundance found in this study was very comparable to other eastern United States coastlines. A study along the length of Georgia's coastal Georgia surface waters (~0.3 m water depth) found on average 2 particles/L (Skidaway Institute of Oceanography, 2017). In Florida, there are 263 out of 593 sites from Florida's Microplastic Awareness Project that have in average 1-5 particles/L (Florida Microplastic Awareness Project, 2018). Oppositely, in the same study in Florida, 67 sites were found to have zero microplastics and 281 sites had 6-20 particles/L or greater (Florida Microplastic Awareness Project, 2018). Since methods were kept the same throughout the Florida project, these

differences may be due to variances between sites in human population density, developed or undeveloped areas, wastewater treatment plant number of sites, distance to the nearest river, the size of the watershed, and physical factors (e.g., winds, tides, river discharge). Additionally, findings of the current study were comparable to the Georgia (Skidaway Institute of Oceanography, 2017) and Florida (Florida Microplastic Awareness Project, 2018) studies as fibers were the main type of plastic particle found in all three studies. While not directly comparable, manta trawl water samples consisted of <1.0 to >560 g/km^2 microplastics by weight by area in Chesapeake Bay (Yonkos et al., 2014). The study found statistically significant positive correlations between microplastic concentrations with human population density and proportion of urban/suburban development within watersheds (Yonkos et al., 2014). Additionally, microplastic concentrations peaked at three of four sites shortly after major rain events (Yonkos et al., 2014). Unfortunately, the method extraction efficiency in this study (28-50%) affects the comparison between other studies and vice versa. Ultimately, it is a consistent challenge in microplastic literature to compare between studies as each study has different methods and their contamination levels are influenced by different natural and anthropogenic factors.

Spatial distribution of microplastics in Winyah Bay. From the results of this study, there was no spatial relationship to microplastic abundance as all the sites were statistically the same in both 2016a, 2016b, and 2017 (Table 7) although spatial relationships to microplastic abundance have been found before in other studies (Yonkos et al., 2014; Yu et al., 2018). This suggests that microplastic particles are evenly distributed in the upper region of Winyah Bay and its tributary river mouths. It may be that the close proximity of sampling locations is the cause for this finding. Additionally, it could be the size and

population of the watershed as Yonkos et al. (2014) looks at a watershed more than three times the size of Winyah Bay with 6 times the population. This difference between studies could also be due to the different sample procedures used. Oppositely, an interaction was found between microplastic abundance and sampling date by sampling site for 2016a samples ($F = 5.581$, $p = .028$, $df = 1$). While an interaction is present, there is no clear speculation as to why.

Microplastic levels in Winyah Bay. Data concerning the abundance of microplastic in the Winyah Bay area are limited to two studies thus far, one of which conducted by Gray et al. (2018) and the other is an ongoing study in Winyah Bay and its tributary rivers by Dr. George Bonellio and Dr. Jane Guentzel at Coastal Carolina University. Gray et al. (2018) sampled for microplastic particles within intertidal shoreline surface sediment and the sea surface microlayer in Winyah Bay from June to August 2014 and found on average 221 particles/m^2 and 30 particles/L, respectively. Some reasons as to why Gray et al. (2018) found more microplastic particles than the current study may be due to (1) the sampling technique, (2) the water layer sampled, (3) the volume of input of each particle type found and the annual/seasonal input of the particle, (4) the sampling location in Winyah Bay, and (5) the low wave energy of Winyah Bay. Each reason is further explained below by corresponding numbers. (1) The particle recovery rates of the current study were between 28-50% showing that our data underestimates the actual microplastic contamination levels in Winyah Bay, while Gray et al. (2018) did not report recovery rates for their method in sampling the surface microlayer. (2) The sea surface microlayer has shown to have more microplastic contamination than underlying surface waters and this is thought to be due to the low density of microplastics in comparison to saltwater (Anderson et al., 2018). This

study found that microplastic types were statistically different from each other based on salinity and temperature of the sample water (Table 4). This finding suggests that particle accumulation throughout the water column is influenced by the difference between the density of the particle and of the chemistry of the surrounding water. (3) This current study found a dominance of microplastic fibers (90%) during summer and fall seasons of 2016 and 2017, while Gray et al. (2018) found a dominance of microplastic fragments (90%) thought to be tire particles during summer of 2014. The typical density of tire rubber ranges from 570 kg/m^3 to 730 kg/m^3 (Federal Highway Administration, 2016) which is less than the typical density of freshwater causing them to float on top of the surface microlayer. Additionally, it may be that sources inputting black fragments reduced input after 2014 and sources inputting fibers heighted thereafter. (4) The majority of the sample sites of the current study were landward of the estuary turbidity maximum zone, while the majority of Gray et al. (2018) sample sites were seaward of the turbidity maximum zone (ETM, further discussed below in "*Microplastic transport and fate into sediments*") resulting in different hydrodynamic conditions. (5) Lastly, the low wave energy of Winyah Bay may keep microplastics in the surface microlayer from mixing with the surface layer underneath it, retaining more particles in the microlayer.

Microplastic transport in Winyah Bay. Our 2017 data showed that microplastic abundance increased as salinity, density, or tidal range increased. Since the water level variance (i.e., tidal range) is mostly explained by the tide and microplastic concentrations increase with increasing salinity it is probable that the main source of microplastic contamination to surface waters (upper ~0.3 m) in Winyah Bay is the coastal zone as the tides bring in water from the estuary mouth into the bay. Another possible source of

microplastic particles to the surface waters of Winyah Bay may be from the resuspension of sediment and mixing of water by the incoming tide. Considering Gray et al. (2018) finding of black fragments in the sea surface microlayer of Winyah Bay and the lack of black fragments found in the surface water samples of the present study, it is unlikely that significant mixing is occurring between the surface water and sea surface microlayer. Rather, elevated microplastic numbers sourcing from water mixing must come from lower in the water column (i.e., tidal wedge) and possibly from those in the sediment. In addition to tides, the microplastic contamination profile of Winyah Bay seems to be heavily driven by microplastic characteristics as the surface waters (~0.3 m) were dominated by microplastic fibers and the sea surface microlayer (~1 mm) and intertidal sediment (~2 cm) were dominated by microplastic fragments. Additionally, the size of the particle was found to have a slight (all $r < .10$) negative linear correlation to salinity, temperature and density of the water, respectively, which would mean as these variables increases the size of the particle decreases. This small correlation explains less than 10% of the variation seen in microplastic abundance, therefore, it is not mechanically important and probably does not have a noticeable impact on the transport of microplastic particles. It may have only been found due the large sample size ($n = 1357$). Correlation outcomes may be slightly skewed as 81% of the particles in this study were between 0.063-2 mm in size. If the length of particles found in this study were more evenly distributed across the 0.063-5 mm spectrum, then maybe there would be a different result that we are unable to see with the majority of particles on the lower end of the scale.

Microplastic sources in Winyah Bay. The sources inputting microplastic particles into Winyah Bay determine the size of the particle found. Our study shows an exponential

decay relationship in the microplastic sizes found (Figure 6) and it may deserve further exploration in a future study specifically leaning on the sources inputting microplastic particles to the area. Considering the majority of our particles were fibers, likely sources are washed/dried clothing articles, rope from marinas, fishing line, or any macroplastic items that have broken down into microplastic. In Georgetown, SC there is one harbor and various craft industries located on the Sampit River and a marina located on the mouth of the Pee Dee River. Additionally, there is one municipal sewage treatment plant that empties into the Sampit River and agricultural lands that provide runoff into all tributary rivers of Winyah Bay. Lastly, Winyah Bay ultimately receives the effluent of 15 total National Pollutant Discharge Elimination System (NPDES) sites (S.C. Department of Health and Environmental Control, 2011). NPDES sites are point sources of pollution which are required by the United States' Clean Water Act to hold a permit for pollution regulation. All the above surely contribute as local sources of microplastics to Winyah Bay. Regional sources of microplastic particles to the area may be attributed with the positive correlation between microplastic abundance and precipitation. As the region receives precipitation, the water, sediment and microplastics from upstream are sent to Winyah Bay. The influx of plastic and trash is very visible even weeks after a hurricane comes through.

Microplastic transport and fate into sediments. The Winyah Bay drainage basin sends an average of 0.43×10^6 ton/year of suspended sediments to its estuary (Patchineelam and Kjerfve, 2004). About 80% of fine-grained sediment that reached Winyah Bay either accumulated in adjacent marshes or estuarine mud flats, and the remaining 20% makes it to the coast or further offshore (Patchineelam et al., 1999). It may be found that the majority of microplastic also accumulates in the adjacent marshes or estuarine mud flats of Winyah

Bay like fine-grained sediment does since microplastics and fine-grain sediment are similar in size and hydraulic behavior. However, it may also be that microplastics do not deposit in similar locations where fine-grain sediment deposit in due to their differences in density and shape. The sampling sites of this study were selected to shed light on the processes controlling suspension and deposition of microplastic particles within Winyah Bay. Unfortunately, with the timing restriction of a master's project and the amount of time it took to establish a method that would work with the composition of the sample, few test sediment samples were processed and there was not sufficient time to perform a method accuracy test. Some of these sample sites are located around the estuary turbidity maximum (ETM), a region where the concentration of suspended sediment may be a hundred times greater than concentrations both seaward and landward. This maximum is formed by tidal processes and the salt front (Patchineelam and Kjerfve, 2004). The ETM may be an accumulation zone for microplastics as, like sediments, they are deposited and resuspended into the water column. Patchineelam et al. (1999) showed through grain-size analyses that clay and silt size particles were predominate in the upper bay by more than 80% (Patchineelam et al., 1999). Later in 2004, Patchineelam and Kjerfve showed that river-borne sediments accumulate in this upper bay region. It's probable that this same region in the upper bay is an area where microplastics accumulate due to their similar size to fine-grained particles, however, density differences may lead to differentiated accumulation zones.

Limitations of the study. The samples in this study are snapshots of microplastic contamination levels in Winyah Bay and they do not incorporate the time scales of possible transport drivers (i.e., river discharge and wind). Cumulatively, these drivers shift in

intensity on daily, weekly, monthly, seasonally and annually scales and possibly creating signatures through microplastic abundances. For example, the sampling months in this study consisted of mean river discharge levels between 125-300 m^3/s (i.e., 4250 to 10250 ft^3/s) and over the past 15 years mean river discharge in Winyah Bay has ranged from -28.32 m^3/s to 3681.19 m^3/sec (i.e., -1000 ft^3/s to 130000 ft^3/s). A future study might be able to find a stronger relationship between mean river discharge and microplastic abundance if samples were taken during the upper and lower ranges of the mean river discharge in Winyah Bay. Also, the distance between the study's sampling sites in Winyah Bay and the USGS sensors at Bucksport is a concern for truly representing interactions between variables and microplastic abundance. Being upriver, Bucksport's microplastic contamination is more influenced by river discharge than Winyah Bay and Winyah Bay's microplastic contamination is more influenced by the tide from its estuarine environment. Future studies would benefit for a closer network of sensors to sampling sites in Winyah Bay. Additional limitations of this study include method recovery rates, procedure consistency with other studies, the multiple methods between samples used in this study, and by the unknown microplastic composition. Preparation-related particle recovery rates were between 28-50% and showed that our data underestimates actual microplastic contamination levels in Winyah Bay. With higher microplastic contamination in Winyah Bay than what our study can show, future studies in Winyah Bay and method advancement are important for its ecological and societal health. The procedures used in this study are not consistent with other studies as they cater to the high organic matter and large fine sediment fraction and jelly-like muds of Winyah Bay. Also, water sampling and processing procedures were not consistent throughout the study requiring microplastic concentration

conversion that may not accurately represent the microplastic contamination levels and the interpreted influence of driving factors on the levels. Lastly, if FTIR were incorporated into the study, it would have had provided the chemical composition of the particles which would allow us to (1) understand microplastic sources better and (2) verify that particles were indeed plastic rather than natural particles mistaken for plastic.

Conclusion

Results from this study reveal the steady occurrence of microplastic contamination in Winyah Bay, SC and the dominate drivers in particle transport within the study area. The majority of microplastic particles found in this study were fibers, and surrounding contamination sources (i.e., marinas, sewage outflow pipes, and NPDES) could influence fiber abundance in this area. The data in this study shows trends and tendencies between microplastic contamination and tides (i.e., salinity, density, and tidal range) and precipitation over time. The data only spans a very narrow discharge range and as a result, the role of river discharge is not fully evaluated here. The data also needs more samples under one consistent method to determine other possible transport factors (i.e., river discharge, wind speed, and wind origin)

Microplastic sampling and procedures for Winyah Bay, an area with a large fine sediment fraction as well as a large organic matter load, were advanced in this study. We found that using a sieve for water sampling reduces contamination within a sample and is a more efficient methodology than using a plankton net. With more research in areas like Winyah Bay with highly cohesive fine sediment, the measurement tools and analysis of the microplastic scientific community will grow allowing for a better understanding of these systems. With method advancement also leading to a highly required consistency and comparability between studies, it will be easier to compare and understand different geographic areas and their physical processes as they all influence the transport and intensity of microplastics differently.

Further research would help to support the findings of this study by exploring each physical parameter in depth to help underline the timing and significances of dominance.

To increase the retention rate of the methods used in this study and to reduce the similarity of blank values to sample values, the water volume sampled should be increased. With a possible near future dredging of the Goat Island river loop, monitoring on microplastic contamination in this area will be extremely valuable in understanding the new implications this action will have on the bay. Even further, resuspension of microplastic contamination during dredging will greatly increase the potential for harm on the present estuarine and coastal ecosystems. A complementary study to this work would be an important extension of the research of how the Winyah Bay food web is currently impacted by microplastic particles and how this might change with future dredging. Considering the high economic importance of the water quality for activities in this area like oyster and shrimp harvesting, more research is necessary to find the impact that microplastic contamination has on these activities. Even further, research is needed to understand microplastic particles as a transport method for plastic additives and hydrophobic chemicals through the environment, consumption by organisms, and the fate of such additives and chemicals (i.e., possibly human digestive tracts from consumption).

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Annex

Table 10. List of all samples by date, name and microplastic abundance in (6.6L, 20L, 1L or m^3).

Method	Date	Sample	Counts	Counts-blank	mp/1L	mp/m^3
2016a	Aug-16	PD2	7	4	0.606061	606.0606
2016a	Aug-16	PD6	9	6	0.909091	909.0909
2016a	Aug-16	PD8	6	3	0.454545	454.5455
2016a	Aug-16	PD10	18	15	2.272727	2272.727
2016a	Aug-16	S4	24	19	2.878788	2878.788
2016a	Aug-16	Wacc4	43	41	6.212121	6212.121
2016a	Aug-16	Wacc6	18	16	2.424242	2424.242
2016a	Aug-16	Wacc8	11	10	1.515152	1515.152
2016a	Aug-16	Wacc10	9	8	1.212121	1212.121
2016a	Oct-16	PD1	20	18	2.727273	2727.273
2016a	Oct-16	PD4	10	8	1.212121	1212.121
2016a	Oct-16	PD5	13	11	1.666667	1666.667
2016a	Oct-16	PD6	15	13	1.969697	1969.697
2016a	Oct-16	PD7	10	8	1.212121	1212.121
2016a	Oct-16	PD8	6	4	0.606061	606.0606
2016a	Oct-16	PD9	20	18	2.727273	2727.273
2016a	Oct-16	PD10	7	5	0.757576	757.5758
2016a	Oct-16	Wacc1	6	3	0.454545	454.5455
2016a	Oct-16	Wacc2	17	14	2.121212	2121.212
2016a	Oct-16	Wacc4	9	6	0.909091	909.0909
2016a	Oct-16	Wacc5	12	9	1.363636	1363.636
2016a	Oct-16	Wacc6	3	0	0	0
2016a	Oct-16	Wacc7	13	10	1.515152	1515.152
2016a	Oct-16	Wacc8	13	10	1.515152	1515.152
2016a	Oct-16	Wacc9	7	3	0.454545	454.5455
2016a	Oct-16	Wacc10	15	11	1.666667	1666.667
2016b	Jul-16	PD1	46	46	6.969697	6969.697
2016b	Jul-16	PD2	40	40	6.060606	6060.606
2016b	Jul-16	PD3	45	45	6.818182	6818.182
2016b	Jul-16	PD4	61	61	9.242424	9242.424
2016b	Jul-16	PD5	101	101	15.30303	15303.03
2016b	Jul-16	Wacc2	28	28	4.242424	4242.424
2016b	Jul-16	Wacc3	26	26	3.939394	3939.394
2016b	Jul-16	Wacc4	26	26	3.939394	3939.394
2016b	Jul-16	Wacc5	21	21	3.181818	3181.818
2016b	Aug-16	PD1	59	56	8.484848	8484.848
2016b	Aug-16	PD3	44	41	6.212121	6212.121

2016b	Aug-16	PD5	43	40	6.060606	6060.606
2016b	Aug-16	PD7	30	27	4.090909	4090.909
2016b	Aug-16	PD9	35	32	4.848485	4848.485
2016b	Aug-16	S1	46	45	6.818182	6818.182
2016b	Aug-16	S2	69	68	10.30303	10303.03
2016b	Aug-16	S3	29	28	4.242424	4242.424
2016b	Aug-16	S5	25	20	3.030303	3030.303
2016b	Aug-16	Wacc3	81	79	11.9697	11969.7
2016b	Aug-16	Wacc5	20	18	2.727273	2727.273
2016b	Aug-16	Wacc7	32	31	4.69697	4696.97
2016b	Aug-16	Wacc9	28	27	4.090909	4090.909
2017	9/9/2017	GS1	17	14	0.7	700
2017	9/9/2017	GS2	11	8	0.4	400
2017	9/9/2017	GS4	31	28	1.4	1400
2017	9/9/2017	GS5	6	3	0.15	150
2017	9/9/2017	GS6	17	14	0.7	700
2017	9/9/2017	GS7	5	2	0.1	100
2017	9/9/2017	GS8	7	4	0.2	200
2017	9/9/2017	GS8dup	8	5	0.25	250
2017	9/16/2017	WR1	10	9	0.45	450
2017	9/16/2017	WR2	5	4	0.2	200
2017	9/16/2017	WR3	5	4	0.2	200
2017	9/16/2017	WY1	6	5	0.25	250
2017	9/16/2017	PD1	4	3	0.15	150
2017	9/16/2017	PD2	4	3	0.15	150
2017	9/16/2017	WY2	5	4	0.2	200
2017	9/16/2017	WY2dup	11	10	0.5	500
2017	9/16/2017	WY3	11	10	0.5	500
2017	9/16/2017	WY4	9	8	0.4	400
2017	9/23/2017	WY5Sam	3	0	0	0
2017	9/23/2017	WY5Jess	6	3	0.15	150
2017	9/23/2017	GS10kar	5	2	0.1	100
2017	9/23/2017	GS10dam	10	7	0.35	350
2017	9/23/2017	GS11jul	4	1	0.05	50
2017	9/23/2017	GS11Kait	10	7	0.35	350
2017	9/23/2017	GS12sam	2	0	0	0
2017	9/23/2017	GS12nat	4	1	0.05	50
2017	9/23/2017	GS13molly	12	9	0.45	450
2017	9/23/2017	GS13kar	10	7	0.35	350

Table 11. Correlation values between 2016a microplastic abundance and salinity, temperature, density, wind origin, wind speed, precipitation, river discharge, and tidal range. Correlation values are listed as r, p-values are p, and sample number is n. Bolded values indicate the correlation is significant. Correlation is significant at the 0.05 level (2-tailed) if noted with * and at the 0.01 level (2-tailed) if noted with **.

		mp/m^3	Salinity (ppt)	Temp °C	density of water (kg/m3)	precip(cm) (3 months preceding)	wind speed (kph)	Dis (m3/s)	tidal range (m)
mp/m^3	r	1	.112	.268	-.010	-.249	.215	-.296	.248
	p		.586	.186	.959	.219	.291	.142	.221
	n	26	26	26	26	26	26	26	26
Salinity (ppt)	r	.112	1	.206	**.895****	-.204	.380	**-.401***	.279
	p	.586		.312	.000	.318	.056	.042	.167
	n	26	26	26	26	26	26	26	26
Temp °C	r	.268	.206	1	-.253	**-.984****	-.007	**-.956****	**.986****
	p	.186	.312		.213	.000	.973	.000	.000
	n	26	26	26	26	26	26	26	26
density of water (kg/m3)	r	-.010	**.895****	-.253	1	.248	.380	.040	-.175
	p	.959	.000	.213		.222	.056	.846	.394
	n	26	26	26	26	26	26	26	26
precip(cm) (3 months preceding)	r	-.249	-.204	**-.984****	.248	1	-.095	**.963****	**-.994****
	p	.219	.318	.000	.222		.646	.000	.000
	n	26	26	26	26	26	26	26	26
wind speed (kph)	r	.215	.380	-.007	.380	-.095	1	-.155	.074
	p	.291	.056	.973	.056	.646		.450	.720
	n	26	26	26	26	26	26	26	26
Dis (m3/s)	r	-.296	**-.401***	**-.956****	.040	**.963****	-.155	1	**-.981****
	p	.142	.042	.000	.846	.000	.450		.000
	n	26	26	26	26	26	26	26	26
tidal range (m)	r	.248	.279	**.986****	-.175	**-.994****	.074	**-.981****	1
	p	.221	.167	.000	.394	.000	.720	.000	
	n	26	26	26	26	26	26	26	26

Table 12. Correlation values between 2016b microplastic abundance and salinity, temperature, density, wind origin, wind speed, precipitation, river discharge, and tidal range. Correlation values are listed as r, p-values are p, and sample number is n. Bolded values indicate the correlation is significant. Correlation is significant at the 0.05 level (2-tailed) if noted with * and at the 0.01 level (2-tailed) if noted with **.

		mp/m^3	Salinity (ppt)	Temp °C	density of water (kg/m3)	precip(cm) (3 months preceding)	wind speed (kph)	Dis (m3/s)	tidal range (m)
mp/m^3	r	1	.165	.082	.156	-.019	.074	.094	-.064
	p		.462	.717	.488	.934	.744	.679	.779
	n	22	22	22	22	22	22	22	22
Salinity (ppt)	r	.165	1	**-.485***	**.999****	**.545****	-.107	**-.480***	.382
	p	.462		.022	.000	.009	.635	.024	.079
	n	22	22	22	22	22	22	22	22
Temp °C	r	.082	**-.485***	1	**-.526***	**-.780****	**.590****	.332	-.105
	p	.717	.022		.012	.000	.004	.132	.643
	n	22	22	22	22	22	22	22	22
density of water (kg/m3)	r	.156	**.999****	**-.526***	1	**.573****	-.137	**-.484***	.376
	p	.488	.000	.012		.005	.544	.023	.084
	n	22	22	22	22	22	22	22	22
precip(cm) (3 months preceding)	r	-.019	**.545****	**-.780****	**.573****	1	**-.675****	-.396	.107
	p	.934	.009	.000	.005		.001	.068	.634
	n	22	22	22	22	22	22	22	22
wind speed (kph)	r	.074	-.107	**.590****	-.137	**-.675****	1	.096	.113
	p	.744	.635	.004	.544	.001		.670	.616
	n	22	22	22	22	22	22	22	22
Dis (m3/s)	r	.094	**-.480***	.332	**-.484***	-.396	.096	1	**-.953****
	p	.679	.024	.132	.023	.068	.670		.000
	n	22	22	22	22	22	22	22	22
tidal range (m)	r	-.064	.382	-.105	.376	.107	.113	**-.953****	1
	p	.779	.079	.643	.084	.634	.616	.000	
	n	22	22	22	22	22	22	22	22

Table 13. Correlation values between 2017 microplastic abundance and salinity, temperature, density, wind origin, wind speed, precipitation, river discharge, and tidal range. Correlation values are listed as r, p-values are p, and sample number is n. Bolded values indicate the correlation is significant. Correlation is significant at the 0.05 level (2-tailed) if noted with * and at the 0.01 level (2-tailed) if noted with **.

		mp/m^3	Salinity (ppt)	Temp °C	density of water (kg/m3)	precip(cm) (3 months preceding)	wind speed (kph)	Dis (m3/s)	tidal range (m)
mp/m^3	r	1	**.402***	-.202	**.422***	**.411***	.195	.254	**.400***
	p		.034	.303	.025	.030	.320	.192	.035
	n	28	28	28	28	28	28	28	28
Salinity (ppt)	r	**.402***	1	-.181	**.888****	**.625****	**.893****	.088	**.932****
	p	.034		.356	.000	.000	.000	.656	.000
	n	28	28	28	28	28	28	28	28
Temp °C	r	-.202	-.181	1	**-.607****	**-.790****	.046	**-.813****	**-.416***
	p	.303	.356		.001	.000	.818	.000	.028
	n	28	28	28	28	28	28	28	28
density of water (kg/m3)	r	**.422***	**.888****	**-.607****	1	**.866****	**.698****	**.441***	**.946****
	p	.025	.000	.001		.000	.000	.019	.000
	n	28	28	28	28	28	28	28	28
precip(cm) (3 months preceding)	r	**.411***	**.625****	**-.790****	**.866****	1	.365	**.807****	**.767****
	p	.030	.000	.000	.000		.056	.000	.000
	n	28	28	28	28	28	28	28	28
wind speed (kph)	r	.195	**.893****	.046	**.698****	.365	1	-.159	**.773****
	p	.320	.000	.818	.000	.056		.420	.000
	n	28	28	28	28	28	28	28	28
Dis (m3/s)	r	.254	.088	**-.813****	**.441***	**.807****	-.159	1	.241
	p	.192	.656	.000	.019	.000	.420		.217
	n	28	28	28	28	28	28	28	28
tidal range (m)	r	**.400***	**.932****	**-.416***	**.946****	**.767****	**.773****	.241	1
	p	.035	.000	.028	.000	.000	.000	.217	
	n	28	28	28	28	28	28	28	28

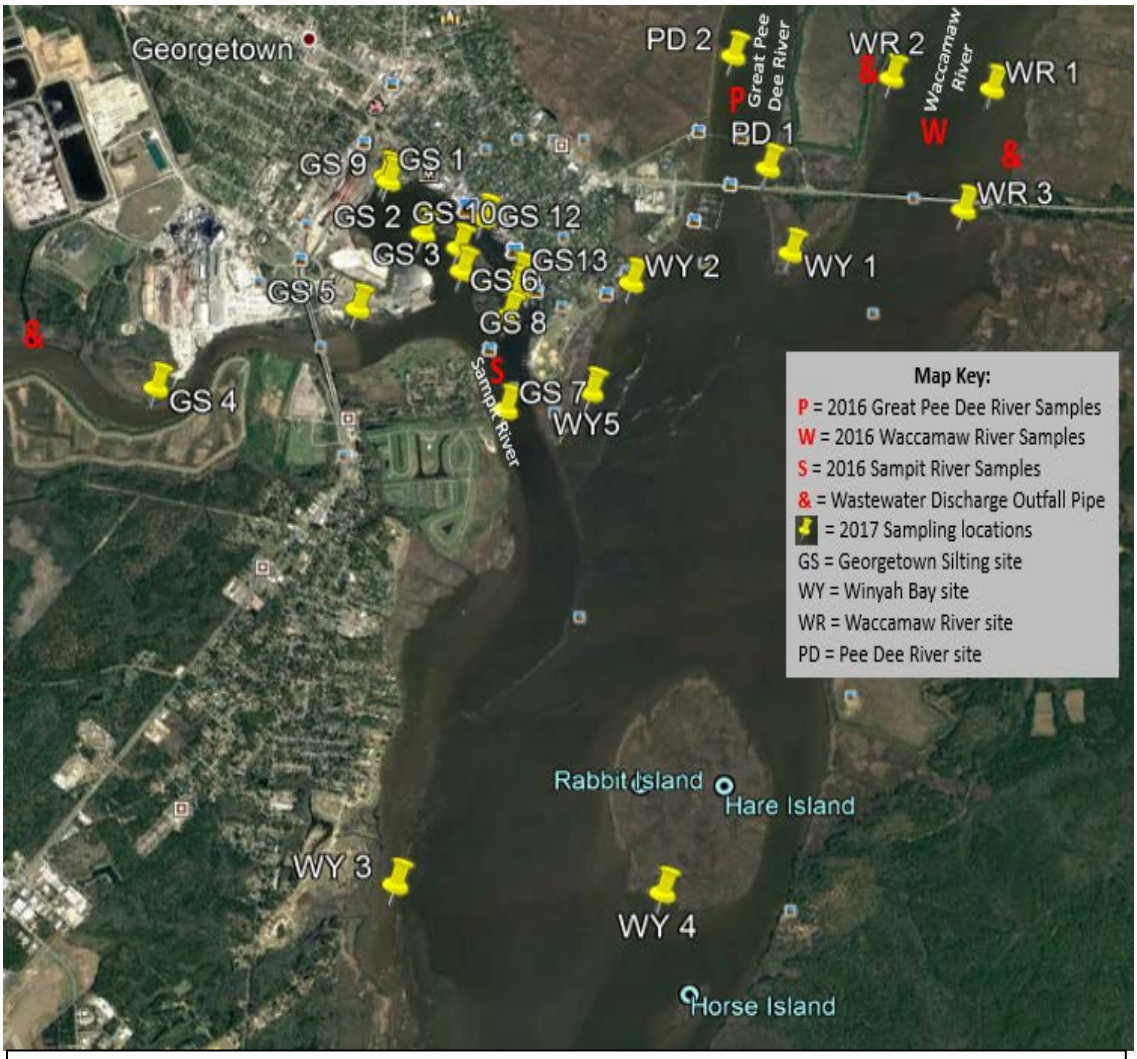

Figure 7. Sample locations by name.

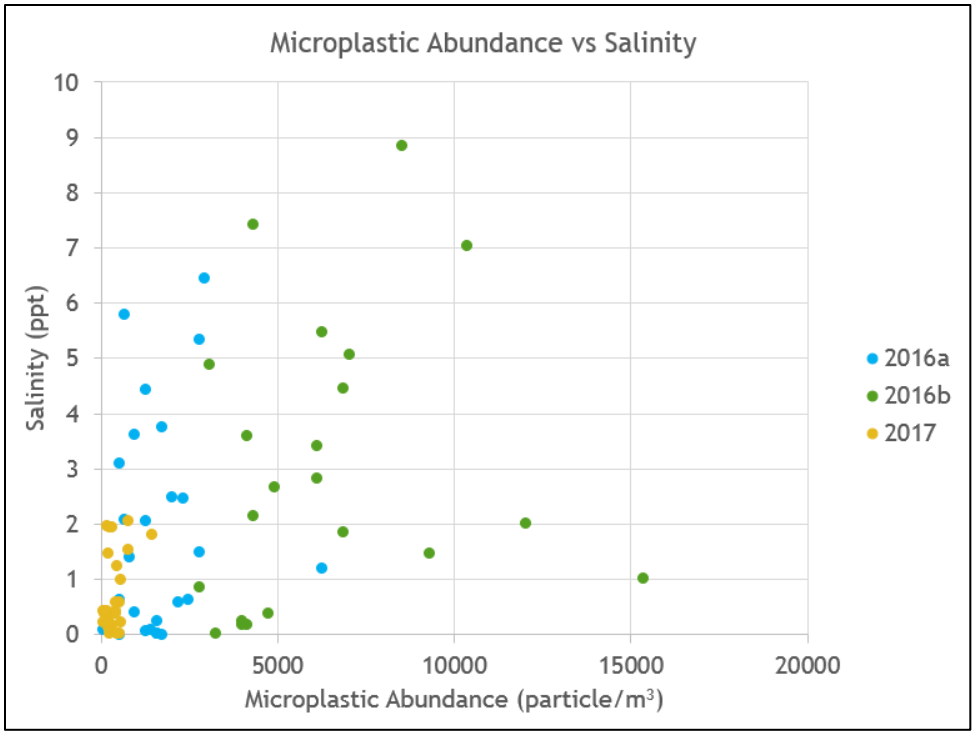

Figure 8. Microplastic abundance on x-axis against salinity on y-axis with 2016a in blue, 2016b in green, and 2017 in orange.

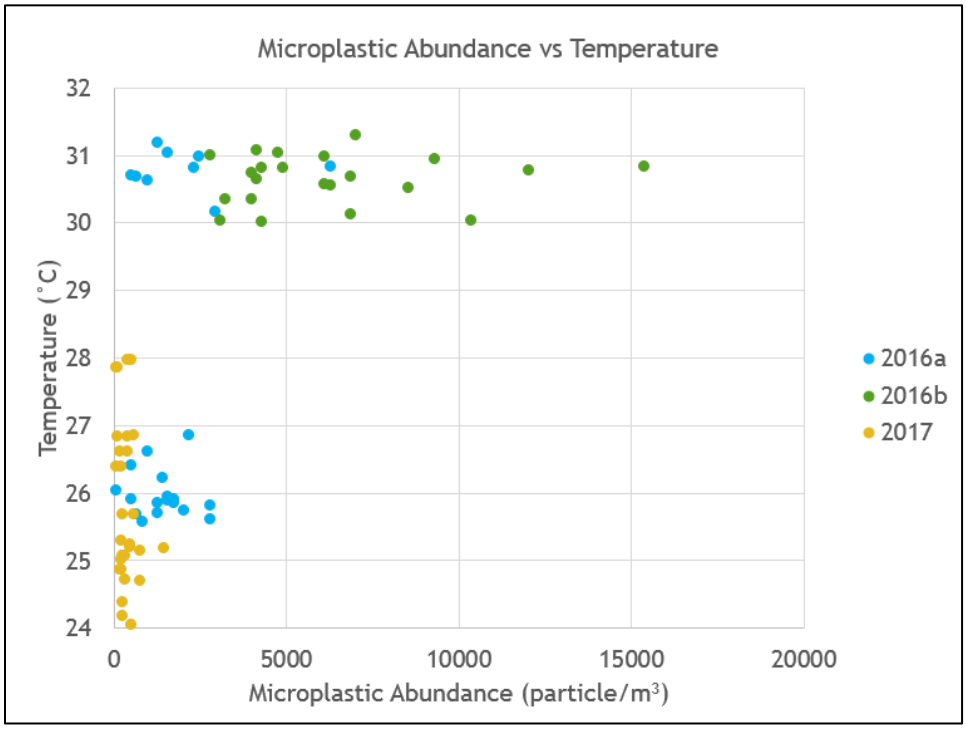

Figure 9. Microplastic abundance on x-axis against temperature on y-axis with 2016a in blue, 2016b in green, and 2017 in orange.

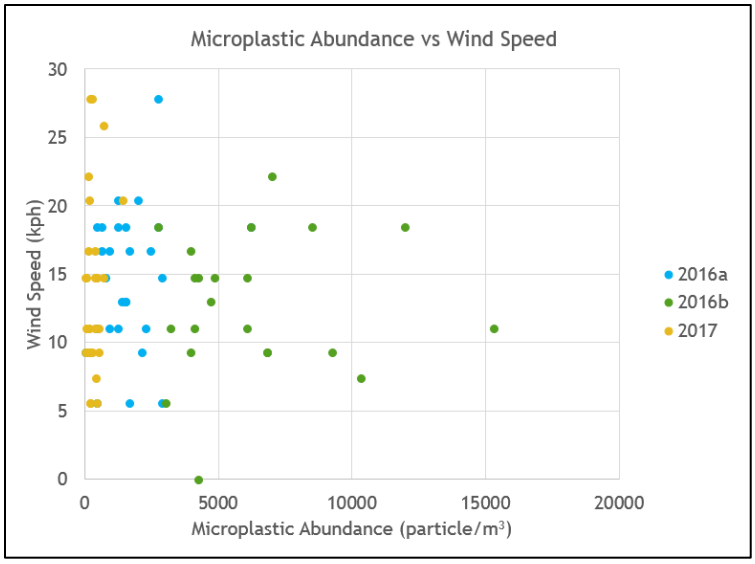

Figure 10. Microplastic abundance on x-axis against wind speed on y-axis with 2016a in blue, 2016b in green, and 2017 in orange.

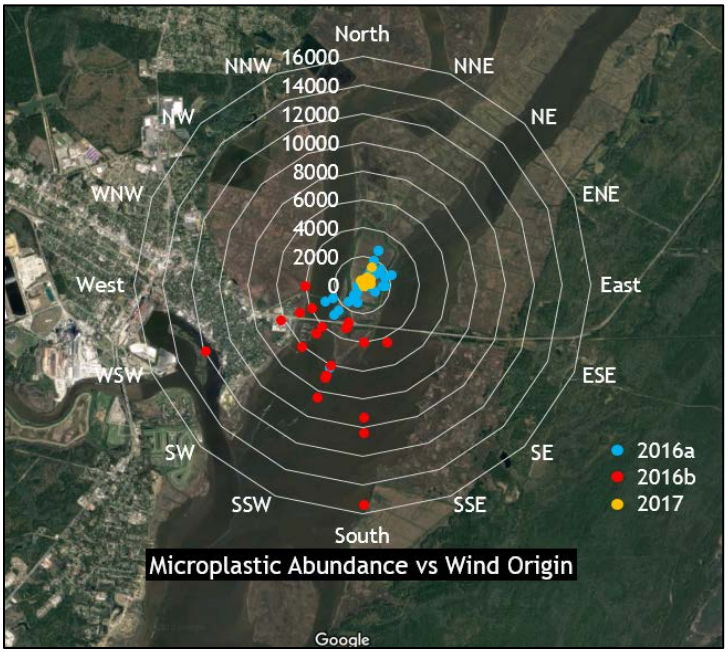

Figure 11. Microplastic Abundance versus Wind Origin. Wind origin indicated by the position of the dot relative to North (0º). Due east is 90 degrees, south is 180 and west is 270 degrees. 2016a in blue, 2016b in red, and 2017 in orange. Each dot is microplastic abundance per sample which increases with distance from the center and is measured in particles/m^3.

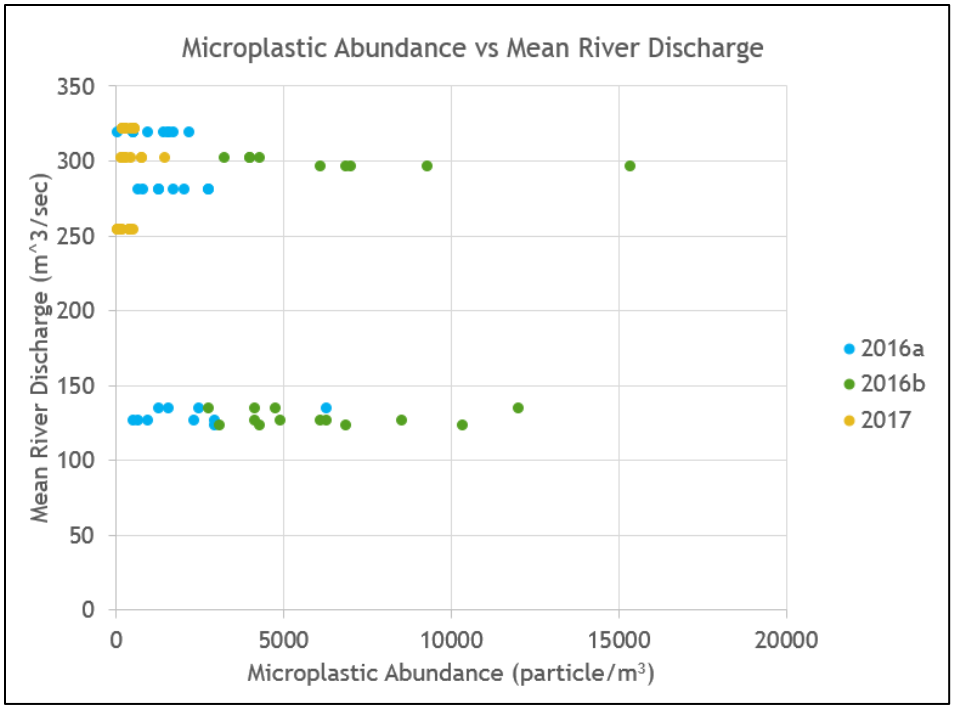

Figure 12. Microplastic abundance on x-axis against river discharge on y-axis with 2016a in blue, 2016b in green, and 2017 in orange.

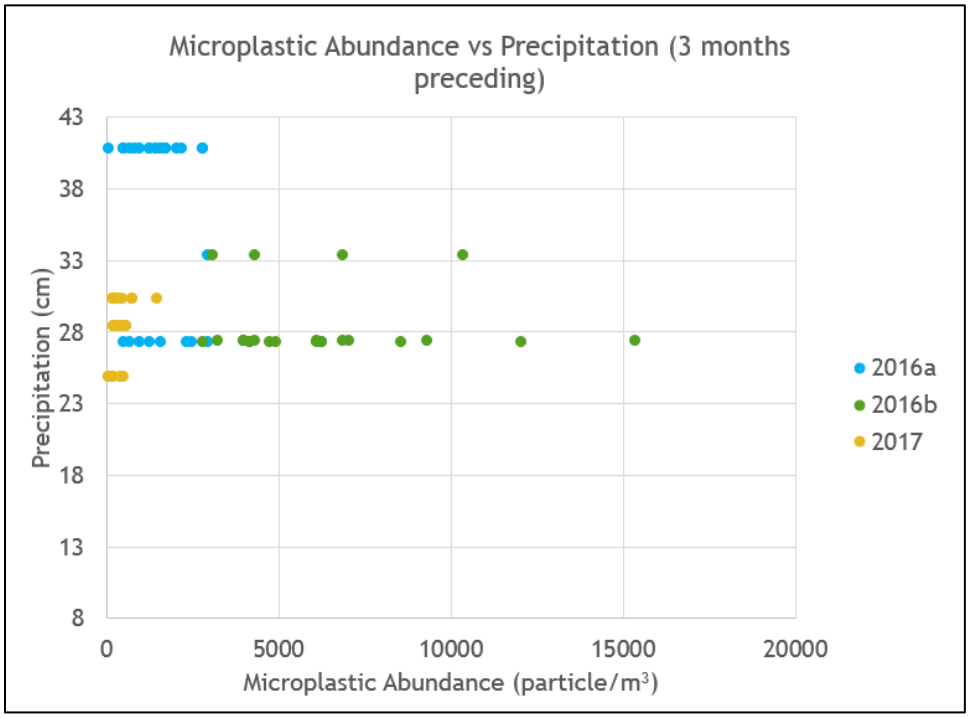

Figure 13. Microplastic abundance on x-axis against precipitation on y-axis with 2016a in blue, 2016b in green, and 2017 in orange.

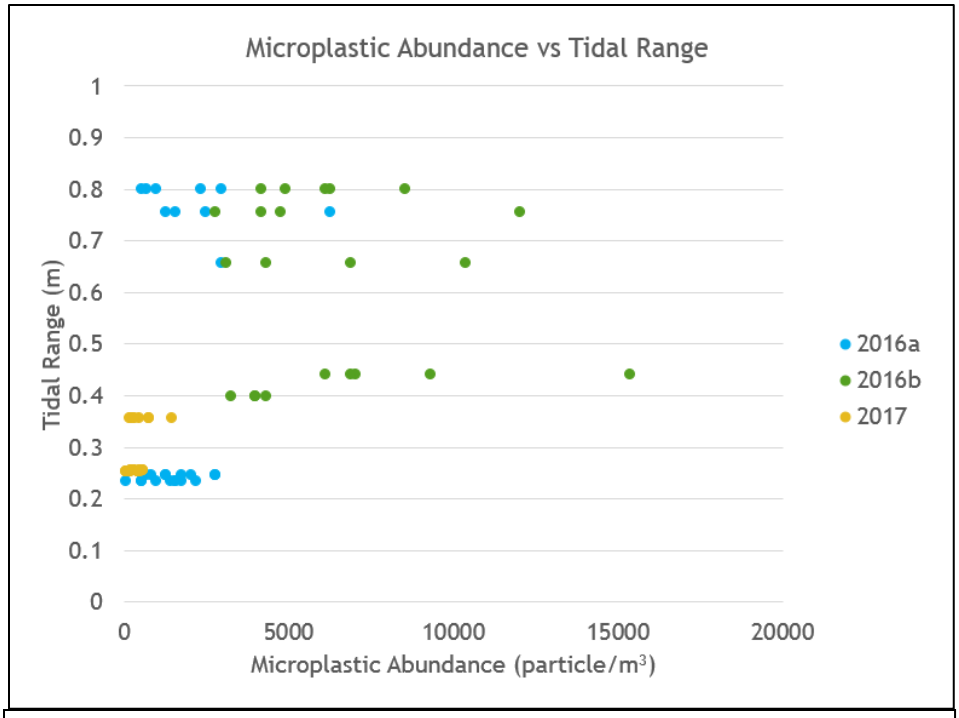

Figure 14. Microplastic abundance on x-axis against tidal range on y-axis with 2016a in blue, 2016b in green, and 2017 in orange.

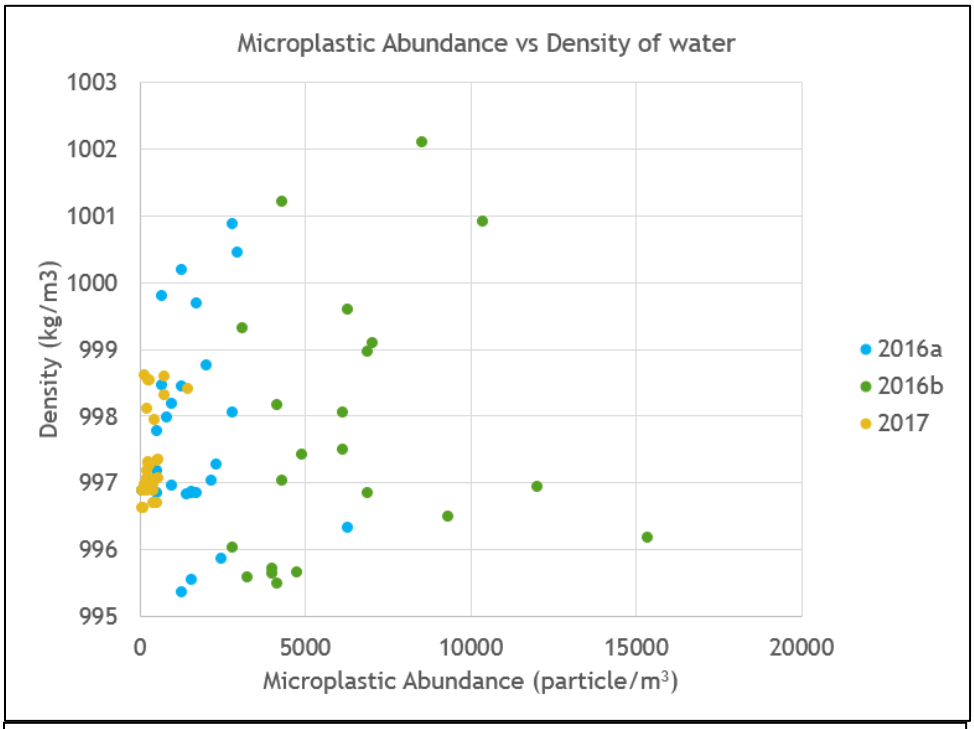

Figure 15. Microplastic abundance on x-axis against density on y-axis with 2016a in blue, 2016b in green, and 2017 in orange.

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Natural Fibers: A Missing Link to Chemical Pollution Dispersion in Aquatic Environments

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NATURAL FIBERS: A MISSING LINK TO CHEMICAL POLLUTION DISPERSION IN AQUATIC ENVIRONMENTS

Marine pollutants caught the United States' public eye in the 1960s creating an environmental movement within the nation that eventually spread worldwide. Marine pollutants include a wide range of materials such as debris (e.g., boating materials, plastic, wood, and glass), hazardous chemicals, human waste, etc. In recent years, one type of marine pollutant, classified as microplastics, has caught special attention, stirred environmentalists, and led to extensive studies over the topics of marine pollution. Microplastics are generally defined as plastic debris with <5 mm in size.[1] Since the late 1990s, many studies revealed the widespread dispersion of microplastics throughout marine environments from surface ocean waters, deep-sea ocean, coastal beaches, and digestion systems of marine organisms. Today, microplastic research is creeping inland looking at their dispersion over rivers, lakes, and terrestrial soil. Approximately 80% of marine microplastic pollution are from on shore through macroplastic degradation and urban wastewater discharge.[1] In particular, cosmetic products containing microplastic "scrubbers" are washed down the drain and their small size enables them to escape from city wastewater treatment plant (WWTP) systems and enter the aquatic environment.[1] Research has revealed that microplastics are a major environmental concern because their large surface areas and molecular characteristics allow sorption of hazardous chemicals, as well as their persistence against degradation and small size provide a special pathway to transfer chemical pollutants through the food chain.[1]

In contrast, studies on the transport of hazardous chemicals by *natural* fibers in aquatic environments are rare compared to those on microplastics. From 2002 to present, the second largest demanded fiber worldwide has been cotton, only after polyester (Table 1).[2] Although produced and used at such a high rate,

Table 1. Comparison of Polyester (Synthetic) And Cotton (Natural) Fibers

type of fiber	demand (million tons per year)[a]	degree of aerobic biodegradation (% C converted to CO_2)[b]	zeta potential (mV) at pH 10[c]
polyester, synthetic	46	13	−69.0
cotton, natural	24	23	−24.5

[a]The world's top two total fibers by demand for 2014.[2] [b]Degree of aerobic biodegradation in natural soil under controlled laboratory conditions after 90 days.[3] [c]Zeta potentials of the cationic surfactant adsorption by fibers at pH 10.[4]

natural fibers have been neglected in marine pollution scientific studies. This is probably due to the commonly held perception that natural fiber's quick degradation does not cause environmental damage. However, clothing fibers from washing machine effluent, like microplastic "scrubbers," can bypass WWTP systems and escape into the aquatic environment.[3] A single garment can produce >1900 fibers per wash,[3] yet this number varies between garments. The time the fibers take to get to estuaries, coastlines, and oceans will also differ depending on river flow speed, weathering, sedimentation of fibers, and ingestion by organisms.

We propose that natural fibers (e.g., cotton) hold environmental concerns like synthetic fibers (e.g., polyester) do, as both have been found to sorb chemical pollutants. Due to the differences in degradability and chemical sorption behaviors between synthetic and natural fibers, they can play different roles in chemical pollution dispersion. Slowly degraded synthetic fibers (Table 1) help keep chemical pollutants unavailable to the surrounding environment for a longer time in comparison to the quickly degrading natural fibers, which

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Figure 16. Screenshot of first page of scientific publication during Master's Thesis (Ladewig et al., 2015).

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Occurrence and distribution of microplastics at selected coastal sites along the southeastern United States

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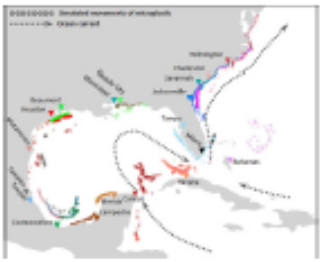
HIGHLIGHTS

- Microplastics were detected from sand samples in each of the sampled Southeastern NPS units, United States.
- Microplastic abundances among sites were highly variable.
- Polyethylene terephthalate (PET) was dominant in the composition of microplastics.
- About 68% of the fibers tested were composed of man-made cellulosic materials such as rayon.
- A Regional Ocean Modeling System model was successfully applied to predict the spatiotemporal distribution of particles.

GRAPHICAL ABSTRACT



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ABSTRACT

To investigate the occurrence and distribution of microplastics in the southeastern coastal region of the United States, we quantified the amount of microplastics in sand samples from multiple coastal sites and developed a predictive model to understand the drift of plastics via ocean currents. Sand samples from eighteen National Park Service (NPS) beaches in the Southeastern Region were collected and microplastics were isolated from each sample. Microplastic counts were compared among sites and local geography was used to make inferences about sources and modes of distribution. Samples were analyzed to identify the composition of particles using Fourier transform infrared spectroscopy (FTIR). To predict the spatiotemporal distribution and movements of particles via coastal currents, a Regional Ocean Modeling System (ROMS) was applied. Microplastics were detected in each of the sampled sites although abundance among sites was highly variable. Approximately half of the samples were dominated by thread-like and fibrous materials as opposed to beads and particles. Results of FTIR suggested that 24% consisted of polyethylene terephthalate (PET), while about 68% of the fibers tested were composed of man-made cellulosic materials such as rayon. Based on published studies examining sources of microplastics, the shape of the particles found here (mostly fibers) and the presence of PET, we infer the source of microplastics in coastal areas is mainly from urban areas, such as wastewater discharge, rather than breakdown of larger marine debris drifting in the ocean. Local geographic features, e.g., the nearness of sites to large

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Figure 17. Screenshot of first page of scientific publication during Master's Thesis (Yu et al., 2018).